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STUDIES ON RHIZOCTONIA SOLANI KÜHN

V. VIRULENCE IN STEAM STERILIZED AND NATURAL SOIL[1]

BY G. B. SANFORD[2]

Abstract

In general, the virulence of *Rhizoctonia Solani* Kühn, shown on potato stems, decreased as the proportion of inoculum, which was grown in steam sterilized soil, increased. Isolates highly pathogenic in a 1 : 15 soil inoculum—natural soil mixture were relatively weak in pure soil inoculum protected from outside contamination.

Sclerotia developed and mycelia massed much more frequently and abundantly in the higher concentrations of soil inoculum than in the 1 : 15 soil-inoculum–natural-soil mixture. Thus, the evidence indicates that conditions favourable to marked vegetative growth of the pathogen tend to depress its virulence. The evidence available suggests that the new and vigorous hyphal growth necessary to sclerotia formation is initiated primarily by high relative humidity of the soil air.

Further evidence was obtained that isolates of *R. Solani* pathogenic to potato may differ greatly in virulence and also in ability to form sclerotia, and also that the *Corticium* stage is an important source of pathogenic and sclerotia-bearing strains of this fungus. Finally, soil-grown inoculum 180 days old was as virulent as that freshly grown.

The method used previously (1, 2) to secure the greatest virulence of *Rhizoctonia Solani* Kühn on potato stems, was to mix thoroughly together, in the proportion of 15 to 1, virgin soil and inoculum of the pathogen grown in steam sterilized soil. In contrast, the virulence of similar inocula of a number of common soil pathogenic fungi, including *Ophiobolus graminis* Sacc., *Helminthosporium sativum* P. K. and B., and *Fusarium* spp., is greatest in a freshly steam sterilized soil.

This paper discusses the pathogenic behaviour of *R. Solani* in natural and steam sterilized soil.

Methods

Soil Inoculum

Ordinary black Edmonton loam (1), with suitable water content, was steam sterilized for three hours in one-litre Erlenmeyer flasks plugged with cotton, and later the particular isolate was grown in these flasks. For a given experiment, this soil inoculum, in the proportion required, was very thoroughly mixed in one pile, with natural virgin, cultivated, or steam sterilized soil. At all times the mixing of the soil and inoculum was done

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under conditions that avoided, as far as possible, additional contamination. The virulence rating of isolates No. 48, 106, 56, and 76, used in these experiments, is in the order mentioned, the first being rather weak, and No. 76 an exceptionally virulent strain.

The Experiment

The water content of the soil was constant throughout each test, being between 29 and 30% of the water-holding capacity. No water was added during a test. Soil temperature for each experiment was satisfactorily constant. With one exception, the tests were carried out at approximately 16°, 17°, or 18° C., and the duration of each was 21 days. Sets of uniform size were cut from tubers of the Early Ohio variety and immediately treated for five minutes in acid mercuric chloride of strength 1 : 1000, then washed in sterile water, and planted. One set was placed on a layer of soil at the bottom of each flask, then covered with the soil-inoculum and soil mixture up to the neck of the flask, approximately five inches. The tubers from which the sets were cut were kept practically dormant at 3° C.

Disease Rating

Each of the 20 plants in a given test was scored for severity of disease, using a scale of 0 to 10. These values, which were later translated into percentages, indicate rather accurately the relative severity of the disease in the various experiments.

Results

Effect on Virulence

The results of the experiments in both cultivated and virgin soil series (Table I) provide convincing evidence that about one part of inoculum with 15 parts of the natural soil used was much more effective than larger proportions of inoculum. In equal parts of inoculum and natural soil the virulence was distinctly reduced.

Of particular interest is the fact that, as a general rule, a uniformly slight amount of disease developed where the potato sprouts grew through the pure soil inoculum protected from outside contamination. For example, the naturally virulent isolate No. 76 produced only relatively slight disease in the pure soil inoculum, and No. 106, which is less virulent, caused little or no disease (Fig. 1). In this particular case no lesions were produced by isolate No. 106, but abundant sclerotia formed, an effect characteristic of this isolate.

It was assumed that the reduced virulence mentioned might be due to certain staling products deposited by the vigorously growing pathogen in the steam sterilized soil. Therefore, data concerning this possibility were obtained in connection with Experiments 9, 11, and 12 (Table I) using isolates No. 106, 56, and 76. In Series A, the soil was inoculated seven days after the set was planted, and in Series B, at the same time. In the first instance the growing sprout from the set and the hyphae usually met midway in the soil, while in the second one the sprout usually emerged into a soil already ramified by the pathogen.

TABLE I

EFFECT ON THE VIRULENCE[1] OF *Rhizoctonia Solani* ON POTATO SPROUTS PRODUCED BY DIFFERENT FRACTIONS OF SOIL INOCULUM

Expt.	Year	Temp. °C.	Isolate	1/16	1/4	1/2	3/4	Soil inoc. A[2]	Soil inoc. B[3]	Soil control
Soil inoculum in a cultivated soil										
1	1933	18	48		17	10	0			Tr.
2	1933	18	76		60	51	24			0
2	1933	18	48		10	1	0			0
3	1933	17	76		55	35	23			Tr.
3	1933	17	48		9	9	7			0
4	1934	18	76		75	81	44			0
4	1934	18	106		50	21	15			0
4	1934	18	48		10	1	0			0
5	1935	16	76		50	45	37			0
5	1935	16	106		43	37	8			0
6	1935	17	76		15	8	5			0
6	1935	17	106		30	5	8			0
6	1935	17	48		15	2	0			0
7	1935	16	76		67	63	38			Tr.
7	1935	16	106		20	14	0			0
7	1935	16	48		7	1	3			0
Soil inoculum in a virgin soil										
8	1934	24	106		15	0	1			0
9	1935	17	106	61				7	0	0
9	1935	17	56	52				6	Tr.	0
9	1935	17	76	58				10	4	0
10	1937	16	76	77	56	49	30	9		0
11	1938	16	76	70				6	1	0
12	1939	16	76	50				8	2	0
Soil inoculum in a steam sterilized soil										
13	1937	16	76		0	5	Tr.			0
14	1940	16	76							0
Steam sterilized soil in a naturally infested cultivated soil										
15	1933	17			67	45	40			72
16	1935	16			20	14	5			27
17	1936	16			5	1	0			11

[1] *Disease rating per cent, based on 20 plants.*
[2] *Steam sterilized soil inoculated seven days after sets were planted.*
[3] *Steam sterilized soil permeated by vigorous growth of pathogen when sprouts emerged from sets.*

The results obtained in the various tests of the two series just outlined, and listed in Table I, indicate that, as a rule, there was definitely less disease and the host more frequently escaped attack when it emerged into soil already permeated by vigorous growth of the pathogen than when host and parasite

FIG. 1. *These photographs illustrate the decrease of virulence and the increase of sclerotia formation by Rhizoctonia Solani in pure inoculum grown in steam sterilized soil. Isolate No. 106 (left) exhibits moderate virulence in a 1 : 15 soil-inoculum–natural-soil mixture, and isolate No. 76 (right) is very virulent.*

met midway in the soil as a result of the delayed inoculation mentioned. However, severity of disease was much reduced and the proportion of plants that escaped attack in either kind of soil inoculum was far greater than in the corresponding series containing 1 part of sterilized soil inoculum and 15 parts of virgin soil.

The addition of steam sterilized soil to a natural soil infested with virulent races of *R. Solani* seems to have somewhat the same tendency to depress virulence as soil-grown inoculum has when added to virgin or cultivated soil (Experiments 15, 16, and 17 (Table I)). Also, the virulence of the soil inoculum of isolate No. 76, when mixed with steam sterilized soil, was very definitely reduced in Experiments 13 and 14.

Effect of Massing of Mycelia and Sclerotia Formation

In inoculated steam sterilized soil, or when large fractions of steam sterilized soil were mixed with virgin soil and inoculated, the formation of sclerotia and the massing of mycelia on the stems were very common and often heavy at normal soil moisture. However, in the 1 : 15 inoculum and natural soil mixture, these formations were much less frequent, and usually not so pro-

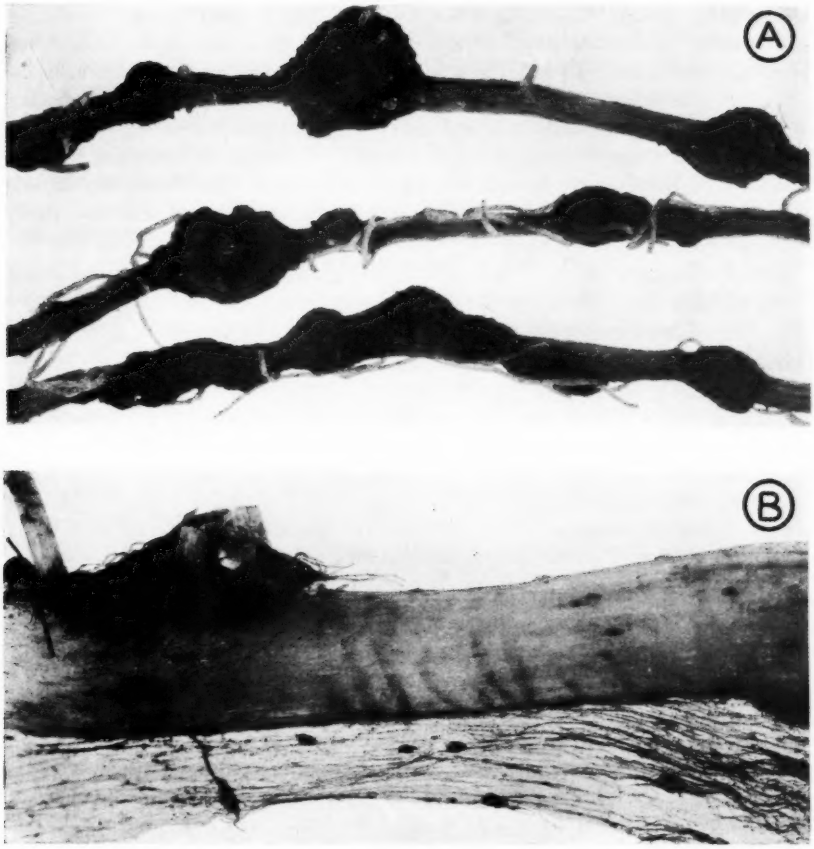

FIG. 2. *These two photographs illustrate how host tissue usually escapes attack when mycelia of Rhizoctonia Solani mass heavily. A. Sclerotia and massing of mycelia of isolate No. 106 on potato roots. B. Weft of mycelia of virulent isolate No. 76 pulled aside. Note young stem has no lesions.*

nounced. Moreover, it was rather exceptional for the mycelia to mass or sclerotia to form on the roots in inoculated natural soil. On the other hand, this sometimes happened in inoculated steam sterilized soil (Fig. 2, A). With other factors constant and favourable for vigorous hyphal growth, the formation of sclerotia was especially favoured when the water content of the soil was slightly above optimum, and as a general rule distinctly inhibited when the soil was rather dry, a fact that agrees with evidence (3) presented earlier. On the other hand, it was found that (2) sclerotia sometimes formed in a soil with a water content of 21% of its water-holding capacity. Although sclerotia formed most readily on the new shoots, the periderm of the parent set provided a substrate almost as favourable.

The evidence indicates that sclerotia formation is very closely associated with sudden growth of new hyphae. Hyphae even a few days old seem to have lost the ability to initiate sclerotia. Very important among the non-living factors are an adequate supply of nitrate nitrogen (3), a high relative humidity in the soil interstices, and a soil temperature range of 16° to 20° C., as demonstrated by this and previous studies. Of these, an optimum relative humidity appears to be the most important single factor. On the other hand, the sclerotia often did not form in the inoculated, steam sterilized loam used, when its water content was optimum for best growth of plants and mycelium.

Certain isolates of *R. Solani* characteristically produce sclerotia much more readily than others under identical conditions. For example, isolates No. 106, obtained from a single basidiospore of the *Corticium* stage, and No. 48, from a sclerotium on a potato tuber, were both far less virulent than isolate No. 76, which was isolated from a disease lesion on a potato stem. Isolates No. 106 and 48 both produced sclerotia and massed hyphae on the shoots much more abundantly and frequently than isolate No. 76.

The marked difference between isolates No. 106 and 76 in ability to form sclerotia, and in pathogenicity under apparently identical conditions, in soil-grown inoculum protected from outside contamination, is shown in Fig. 1. These are typical of the 20 plants in each lot.

Effect of Age of Inoculum on its Virulence

The effect of age and condition of culture has been discussed by several workers without agreement as to whether a young culture is more virulent than an old one. In general, however, these discussions seem to refer to the age of the culture in test tubes rather than to the age of inoculum as prepared in bulk for experiments in the soil. In the absence of satisfactory evidence on this important question, similar experiments were completed during 1937, 1938, and 1939. Each year inoculum of No. 76, a very virulent isolate of *R. Solani*, was increased in sterilized black loam in Erlenmeyer flasks, as described under "Methods". Thus, there were obtained different lots of inoculum, the age of which varied from 6 to 180 days. As soon as the myce-

TABLE II

EFFECT OF AGE OF INOCULUM ON VIRULENCE OF *Rhizoctonia Solani* ON POTATO

Year	Average[1] disease rating, %							Temp., °C.	Soil moisture[2]
	Age of inoculum in days								
	180	150	120	90	60	30	6		
1937	–	60	63	57	57	54	57	17	28
1938	50	60	42	61	60	56	52	17	28.6
1939	–	40	42	41	40	46	40	16	29

[1] *Based on 20 plants, each in separate flask.*
[2] *Per cent moisture holding capacity.*

lium had ramified through the soil in the flasks, which usually required a period of about ten days, that particular lot of flasks was stored at approximately 3° C. until used, and the moisture content maintained. In preparing the experiment, 1 part of this soil inoculum was mixed with 15 parts of virgin black loam. The test was made at 16° C. on the Early Ohio variety grown in flasks as described under "Methods". The disease ratings, arising from the inocula of different ages, are listed in Table II. The evidence is very convincing that the soil-grown inoculum six days old was as virulent as that at least 150 days old.

Discussion

The results of the many experiments listed in Table I leave no doubt that *R. Solani* was much more virulent in the inoculated natural black loam used, with its natural complement of microflora and fertility, than in the same soil steam sterilized and inoculated. The data in Experiments 8, 9, and 10 (Table II) prove conclusively that in general, isolates that are normally very virulent in a natural soil with which has been mixed a small portion of soil inoculum are almost certain to produce a very slight amount of disease in soil-grown inoculum not mixed with natural soil.

As the decrease of virulence closely followed the increase of soil inoculum, one suspects that, for most effective infection, there developed too much mycelium of the pathogen introduced, or too great a growth of it afterwards, as a result of increased fertility made available by sterilizing the black loam, or by staling effects of the more abundant mycelial growth of the pathogen. In this connection, the observational evidence from thousands of young potato plants throughout these studies seems to indicate that the pathogen is most virulent when its hyphae are very young, very thin, and still hyaline, and difficult to find on the susceptible host tissue. Indeed, when the mycelium can be easily seen, and especially when it masses more or less heavily, susceptible host tissue is usually afforded complete protection from infection (Fig. 2, B) despite the fact that the isolate is potentially very virulent. This observation has been so consistent in these experiments during seven years, that one must conclude that factors which cause massing of the mycelium also depress its virulence.

Evidence concerning formation of sclerotia on the underground stems of potato plants indicate that these bodies usually accompany sudden and abundant mycelial growth and that the humidity of the soil air is of prime importance. Also, since sclerotia formed quite readily during this and previous (1, 2) experiments at soil temperatures from 15° to 22° C., when the water content of the soil was about 21 or 28 to 30% of its moisture-holding capacity in both the steam sterilized and the natural soil, it seems clear that conditions favourable for sclerotia are not narrowly restricted by temperature or by water content of the soil within the range mentioned. The relative effect of antagonism on sclerotia formation in the two soils mentioned was not determined.

A possible explanation of the equal virulence of the old and new inocula under the conditions of the test is that infection would depend on new growth from bits of mycelium scattered throughout the soil, and that any initial differences in vigour of growth and virulence would have disappeared by the time the sets had sprouted. Probably one should not expect differences to appear in a test of this kind.

In conclusion, these studies throw an interesting light on the general pathogenicity and virulence of *R. Solani* under field conditions. First, there are the important differences in pathogenicity of the various isolates as determined in this and a previous study (1). These differences undoubtedly range from practically zero to very pathogenic. Isolates also differ in ability to form sclerotia. Being a natural and vigorous soil saprophyte, the various isolates of the pathogen increase or decrease in abundance and relative prevalence, depending on local conditions. It also seems likely that the effect of a given crop on the increase or persistence of the various races in the soil is imperfectly understood. In addition to this general situation, is the fact that fertility, water content, or temperature of the soil may markedly reduce or increase the ability of the various races to successfully attack the host. These aspects of the problem are now being examined further in connection with a study of the effects of various food sources on the virulence and persistence of the pathogen.

References

1. SANFORD, G. B. Can. J. Research, C, 16 : 53-64. 1938.
2. SANFORD, G. B. Can. J. Research, C, 16 : 203-213. 1938.
3. TYNER, L. E. and SANFORD, G. B. Sci. Agr. 16 : 197-207. 1935.

THE RELATION BETWEEN FROST RESISTANCE AND THE PHYSICAL STATE OF PROTOPLASM

II. THE PROTOPLASMIC SURFACE[1]

By D. Siminovitch[2] and J. Levitt[3]

Abstract

Deplasmolysis injury, ductility of cytoplasmic strands, and the shape assumed by injected oil drops on deplasmolysis were investigated. The surface membrane of the protoplast of non-hardy cells stiffened when dehydrated osmotically. As a result, it ruptured readily when subjected to tension. The stiffening either failed to occur in hardy cells, or it arose only as a result of a much greater dehydration (depending on the degree of hardiness). The refractive index of the protoplasmic surface increased more on dehydration in the case of non-hardy than of hardy cells. Plasmolysis, if maintained for some time, induced a clumping of plastids and granules (systrophy) in non-hardy but not in hardy cells. All these facts indicate a greater hydrophily in hardy than in non-hardy cells—both of the surface membrane of the protoplasm and, as shown in Part I, of the protoplasm as a whole, although it is probably less marked in the latter.

In Part I of this series (7) it was shown that the frost-hardening process produces certain changes in the physical state of protoplasm. The methods used, however, revealed the nature either of the protoplasm as a whole or simply of the mesoplasm, and yielded little or no information concerning the ectoplasm. This paper is concerned primarily with the properties of the protoplast surface, as revealed by investigations of deplasmolysis injury, ductility of cytoplasmic strands, and the shape of injected oil drops.

Deplasmolysis Injury

Though it has been established by Scarth and Levitt (8) that hardy cells are more resistant to deplasmolysis injury than are non-hardy cells, more information is required in order to establish the exact conditions under which the differences occur and the exact location of the injury. As in previous investigations, plasmolysis, vital staining (with neutral red), and the general appearance of the cytoplasm were used to determine whether the cells were alive.

Effect of Deplasmolysis Rate

Dehardened cortical cells of *Catalpa* and *Cornus* twigs were progressively plasmolysed in stronger and stronger balanced solutions of sodium chloride and calcium chloride (9 : 1) until an osmotic concentration of 200 atm. was reached. They were then partially deplasmolysed in a solution having a concentration of 50 atm. and were finally transferred to water. Though the protoplasts expanded slowly in the water, they all burst before reaching

[1] *Manuscript received October 22, 1940.*
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the cell wall. This occurred even if deplasmolysis was slowed up still more, by adding water to the plasmolyte drop by drop. If, however, deplasmolysis was not allowed to proceed too far, the protoplasts did not burst.

Hardy cells of *Catalpa* and *Cornus* always deplasmolysed without injury, even when transferred directly to water from the strongest solution.

Similar results were obtained with cane and invert sugar.

Effect of Dehydration and Rehydration in the Absence of Plasmolysis

Cortical sections of *Catalpa* and *Cornus* were transferred directly to very strong sugar solutions (saturated sucrose or supersaturated invert sugar with osmotic pressures of about 200 to 300 atm.). The cells contracted as a whole, the wall collapsing and remaining in contact with the protoplast. This was presumably due to the slow penetration of the sugar molecules into the cell wall. Hardy cells in this state suffered no injury on transfer to water. The protoplasm swelled more or less uniformly on all sides as the whole cell expanded. Non-hardy cells, however, were all killed, except a few in which protoplasts or vacuoles with coagulated protoplasm underwent pseudo-plasmolysis. Even those subsequently succumbed.

These results are summed up in Table I.

TABLE I

SURVIVAL OF DEHARDENED AND HARDY CORTICAL CELLS OF *Catalpa* AND *Cornus* ON TRANSFER FROM HYPERTONIC TO WEAKER SOLUTIONS

Solutes	Osmotic conc. of solutions, atm.	State of cells in strongest solution	Cells surviving	
			Dehardened	Hardy
Sodium chloride + calcium chloride (9 : 1)	50→0	Plasmolysed	Most	All
	50→200→30→0	Plasmolysed	None	All
Invert sugar and sucrose	50→300*→27	Plasmolysed	All	All
	300*→27	Collapsed	Few	All
	27→0	Plasmolysed	All	All
	50→300*→27→0	Plasmolysed	None	All
	300*→27→0	Collapsed	None	All

**Approximate (molar concentration about 6 M).*

Effect of Increase in Surface Area during Deplasmolysis

Sections of dehardened *Catalpa* cortex which were cut directly into *M* sodium chloride and calcium chloride (9 : 1) showed convex plasmolysis in most cells. If, after about half an hour, they were transferred to water, almost all were killed. On the other hand, sections which were first put in hypotonic calcium chloride or dextrose, showed concave plasmolysis on transfer to the above plasmolyte. If they were transferred to water even after more than half an hour in the plasmolyte, most of the cells deplasmolysed quite normally and remained uninjured. Though the dehydration was

exactly the same in both cases, the protoplast surface, being more reduced in the first test, had also to stretch more, and this produced the injury.

Effect of Length of Time in the Plasmolyte

Previous investigations by Scarth and Levitt (8) have shown the importance of the time factor—the longer the sections were left in the plasmolyte, the greater was the deplasmolysis injury. Further tests indicated that hardiness can be correlated with the time necessary to leave the sections in a solution before obtaining deplasmolysis injury. *Hydrangea*, *Picea*, and *Catalpa* cells tested during fall were all killed on deplasmolysis after a 10 minute plasmolysis in a solution of 4 *M* sodium chloride and calcium chloride (9 : 1). In early winter, these three species became more hardy and required exposures of 30, 90, and 120 minutes, respectively, to the same plasmolyte before suffering deplasmolysis injury.

On the other hand, as shown in Part I of this series (7), the difference in resistance to deplasmolysis injury in the case of cabbage was slight if both the hardened and unhardened cells were plasmolysed for only a short time. Since the protoplasts rounded up gradually, the extent to which reduction of surface had proceeded might be a factor in the time effect. Experiments with unhardened cabbage throw some light on this question (Table II).

TABLE II

EFFECT OF TIME IN PLASMOLYTE ON DEPLASMOLYSIS INJURY TO UNHARDENED CABBAGE
(Average of three plants)

Dextrose conc., *M*	Time in plasmolyte	Plasmolysis shape	Cells killed on deplasmolysis, %
1.0	15–20 min.	Mostly concave	25
	2 hr.	Mostly convex	50
	5½ hr.	All convex	90
0.75	15–20 min.	Mostly convex	25
	2 hr.	All convex	40
	5½ hr.	All convex	90

The injury obviously increased with the time in the plasmolyte, even after the protoplasts had completely rounded up. Degree of surface stretch was therefore not a cause of this increased injury. On the other hand, it might be due to a stiffening of the protoplast surface in plasmolysed cells. If deplasmolysis injury is really a surface injury, such a stiffening would explain the time factor. In order to clear up this point, the deplasmolysis process was observed directly.

As already mentioned, a large proportion of cells of unhardened plants, plasmolysed (concavely) in a solution of sodium chloride and calcium chloride with an osmotic concentration of 50 atm. and then transferred to water, were uninjured. Some 25%, however, were killed. Observation of these during deplasmolysis revealed an increase in thickness of the cytoplasm layer followed

by rupture of the outer membrane before deplasmolysis was completed. This allowed escape and dispersion of the protoplasm. The vacuole continued to expand slightly, then it, too, burst. The bursting of the outer membrane occurred either at the side of the protoplast (just where it touches the wall) or at the tip where the cytoplasm layer is thickest.

When the cells were deplasmolysed from stronger solutions there was no apparent swelling of the protoplasm or rupture of the membrane. The cytoplasm layer as a whole appeared to thin out and finally burst. The concentrations of these solutions, however, were much higher than the lowest capable of causing ectoplasmic deplasmolysis injury—especially after one or more hours in the plasmolyte.

These results favour the assumption that the increased deplasmolysis injury resulting from increased time in the plasmolyte is due to a stiffening of the protoplasmic membrane. Table III indicates that this stiffening occurs much more readily in unhardened than in hardened cells.

TABLE III

DEPLASMOLYSIS INJURY TO UNHARDENED AND HARDENED CABBAGE CELLS

Dextrose conc., *M*	Time in plasmolyte, hr.	Degree of plasmolysis (average of 10 cells)	Cells dead after deplasmolysis, %
(a) Unhardened			
0.5	5½	0.67	75
	6	.63	25
	6½	.66	50
(b) Hardened (9 to 12 days at 5° C.)			
2.0	6	0.28	50
	5½	.27	10
	5	.28	50

A concentration of plasmolyte four times as great was required to cause similar injury to hardened as to unhardened cells. At their respective critical concentrations, the hardened cells were reduced to less than one-third their normal volume, the unhardened to only two-thirds. It is interesting to note that at their critical freezing temperatures the hardened cells are also reduced to less than one-third their normal volume, whereas the unhardened are reduced to one-half. On the other hand, when deplasmolysis injury was determined 15 to 20 min. after immersion in the plasmolyte, the unhardened cells suffered the same injury (about 50%) only when a concentration of 1.5 *M* dextrose was used. In this solution their volume was reduced to less than one-third normal (about the same as the hardened in 2.0 *M* dextrose). The hardened cells, however, were injured to about the same extent whether in the plasmolyte for one-quarter hour or six hours and in both cases were reduced to the same volume as at their critical freezing

temperature. The unhardened cells at their critical freezing temperature were reduced to a volume midway between those that are critical for deplasmolysis injury after one-quarter hour and after six hours in the plasmolyte.

Similar tests were made with hardy and dehardened *Catalpa* twigs. A modification in the technique enabled the cells to round up even in the extremely concentrated solutions that were used for the hardy cells. (It should be mentioned that this method does not work with all plants). Sections of cortex were placed in 3 to 6 cc. of slightly hypotonic dextrose in a watch glass, which was left exposed to the dry atmosphere (about 20% relative humidity) of the laboratory. From time to time as the solution gradually became concentrated, a section was removed and transferred to water. When plasmolysis was sufficiently strong to cause death on transfer to water (usually after about 12 to 15 hours) 10 cells in a similar section were measured (Table IV).

TABLE IV

DEPLASMOLYSIS INJURY IN HARDY AND DEHARDENED *Catalpa* CELLS

Dehardened		Hardy	
Degree of plasmolysis	Injury, %	Degree of plasmolysis	Injury, %
0.53	0	0.29	0
.51	50	.28	0
.46	100	.28	0

The hardy cells were not injured even when the solution became supersaturated (about 6 *M*) and their volume was reduced to less than one-third normal; the cells were still alive even two or three days after the test. The dehardened cells were killed when their volume previous to deplasmolysis was reduced to one-half normal. The concentration of solution causing injury to the dehardened was not determined, but on the basis of previous experiments must have been about molar. The osmotic pressure of the supersaturated solution is about 20 times as great as that of a molar solution, and the dehydrating forces are equal to those produced by a freeze of about $-20°$ and $-2°$ C., respectively. Actually, the hardy cells had withstood $-20°$ C. in nature, while an artificial freeze of $-5°$ C. killed the dehardened.

It is interesting to note that the hardy cells were killed if deplasmolysed in water at $+2°$ C. (instead of $+20°$ to 25° C.) after plasmolysis in supersaturated dextrose at room temperature in the usual way. Furthermore, there was a limit to the length of time the sections could be left in the plasmolyte before deplasmolysis. If this time exceeded about 15 to 20 hours, deplasmolysis from the supersaturated dextrose might cause death. As mentioned above, the dehardened *Catalpa* cells were capable of surviving deplasmolysis from 2 *M* sucrose if placed directly in it and transferred to water a short time (about one-quarter hour) later.

It is evident from these experiments that stiffening of the protoplast surface of non-hardy cells increases greatly with the time in the plasmolyte. In hardy cells there is little change within the times of test. Visual evidence of a difference in non-hardy cells is readily obtained. If sections of dehardened *Catalpa* or *Hydrangea* were plasmolysed in dextrose solutions, the protoplasts rounded up and, either simultaneously or within some time after rounding up, the protoplasm underwent a distinct change (Plate II, *R*). The chloroplasts, granules, and granular kinoplasm all accumulated in one clump either at a free end of the protoplast or as a girdle around the middle. The rest of the protoplast was surrounded only by the hyaloplasm. Sometimes the surface of the protoplast was buckled in, owing to tension on a cytoplasm strand. If the concentration of a plasmolyte was too high, these changes might fail to occur. These phenomena were frequent but not constant in unhardened cabbage cells; they were never seen in the hardened.

It is interesting to note that Gessner (4) has observed this "systrophic" balling in the cells of the water leaves of an amphibious *Ranunculus* but not in those of the air leaves. The former proved to be much more sensitive to drought injury, although it is not clear whether this was due to a morphologic or a physiologic difference.

As to the cause of the clumping, Germ (3) considers it a direct result of plasmolytic stimulation and found that it was favoured by any agent that reduced protoplasmic viscosity. This would indicate that after slight plasmolysis has been maintained for some time, the non-hardy cell possesses a lower protoplasmic viscosity than does the hardy.

Correlation with Varietal Hardiness

For practical purposes, deplasmolysis injury is more likely to serve as a test of hardiness if the membrane stiffening is permitted to occur, since the differences seem greater in this case. In the following tests, the sections were therefore left in the plasmolyte for four to five hours before deplasmolysis.

Four alfalfa varieties (two hardy and two tender) were hardened for two weeks at +5° C. (with continuous light) after growing for four to five months in the greenhouse. Sections of the cortex were then made from the first half-inch of the root. The osmotic concentration of the cell sap was practically the same in all varieties—about 0.4 *M* calcium chloride (25 atm.).

The sections were cut into isotonic or slightly hypotonic dextrose, evacuated to remove the air from the intercellular spaces, and then transferred to a concentrated solution of dextrose (or invert sugar when more than 3 *M* was used).

Both hardy varieties possessed a distinctly greater resistance to deplasmolysis injury than the more tender varieties (Table V). Control plants (six of each) frozen at −11° C. for seven hours were examined two weeks later. All the Arizona and four of the Kansas plants were dead. Hardistan and Grimm were all alive. In all cases the old foliage was dead.

TABLE V

DEPLASMOLYSIS INJURY TO HARDENED ALFALFA VARIETIES. EACH VALUE FOR AN AVERAGE OF THREE PLANTS GROWN IN THE SAME POT

	Variety	Time hardened, days	Conc. of dextrose or invert sugar, M	Injury, %	
Tender	Kansas	11	3.0	75	
		14	3.5	75	
		15	4.0	90	
					Av. 80
	Arizona	11	3.0	100	
		14	3.5	75	
		15	4.0	100	
					Av. 90
Hardy	Grimm	11	3.0	50	
		14	3.5	10	
		15	4.0	50	
					Av. 35
	Hardistan	11	3.0	25	
		14	3.5	0	
		15	4.0	70	
					Av. 30

From all these experiments certain conclusions may be drawn. The rate of protoplast expansion is obviously a negligible factor in deplasmolysis injury. Nor is the injury dependent solely on the degree of surface extension during deplasmolysis, for cells can be killed as a result of collapse without any change in surface area. This fact and the lack of protoplasmic swelling during deplasmolysis after strong plasmolysis, indicate that the injury may be initiated during dehydration. The damage is obviously not an ion effect, since similar results were obtained with non-dissociating sugars. Yet the injury is dependent not only on the degree of dehydration previous to deplasmolysis, but also on the degree of surface extension during the latter process. In some cases at least, the injury is localized in the outer membrane, the bursting of which is followed by death of the cell. The tendency of this membrane to burst on expansion increases with time in the plasmolyte, owing presumably to increased stiffening. But this occurs much more readily in non-hardy than in hardy cells. The protoplasm of non-hardy cells also undergoes a "systrophic" balling. In the protoplasm of hardy cells this does not occur.

Deplasmolysis injury therefore depends on the physical state and the degree of expansion of the protoplasmic membrane, or in extreme cases, of the protoplasm as a whole.

Ductility of Cytoplasmic Strands

When a protoplast plasmolyses convexly, it still retains cytoplasmic connections with its wall, in the form of fine strands that are usually all but invisible. If its cytoplasm is liquid, its consistency must be high to allow the formation of these strands; if solid, it must be ductile. Observations

of the strands under different conditions should therefore yield some information concerning protoplasmic consistency or ductility.

Cortical cells of *Hydrangea* proved very satisfactory for this study, since they possess somewhat more distinct strands than most other cells, probably owing to the large pits with which the strands are presumably (sometimes visibly) connected. Observations were made on the large cells next to the bark. At least some of these from both hardy and dehardened twigs tended to plasmolyse convexly.

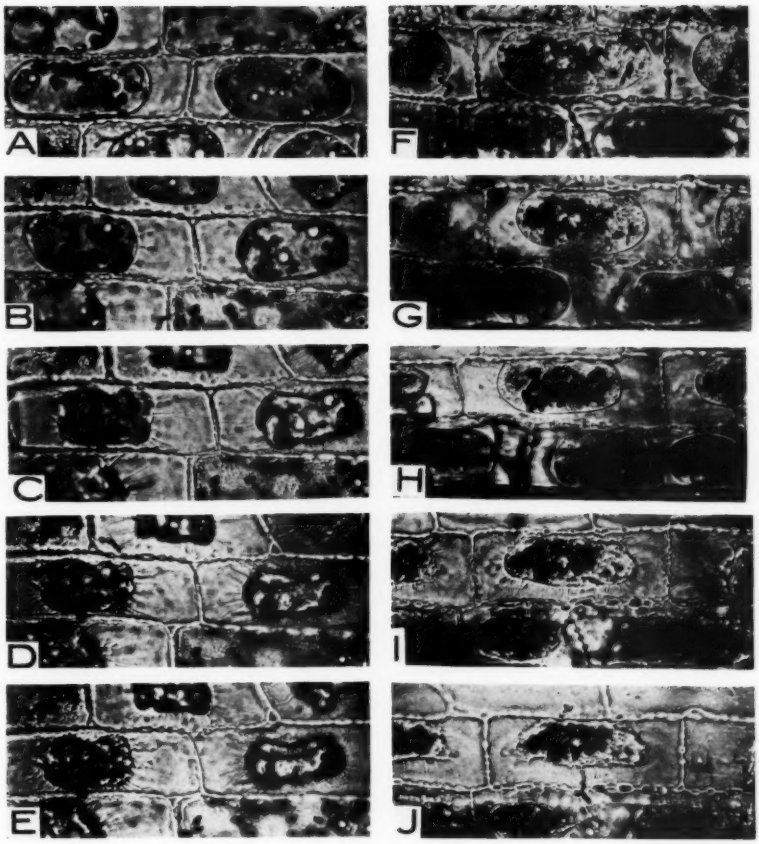
The sections were placed in slightly hypotonic dextrose (0.25 *M* for the dehardened, 0.50 *M* for the hardy) and evacuated to remove the air bubbles in the intercellular spaces. They were then transferred to a hypertonic solution causing shrinkage of the cells to about three-fourths their normal size, and, after about one-half hour, were transferred to a still stronger solution. In this way the same cells were observed in a series of concentrations up to saturated and (in the case of hardy cells) even supersaturated dextrose.

In 0.6 *M* dextrose (and even in solutions of lower concentrations) the dehardened cells showed very fine and barely visible strands (Plate I, *A*). In higher concentrations these became more distinct (Plate I, *B–E*). Since they had been stretched thinner, this could be due only to an increased refractive index resulting from their dehydration. In the case of the hardy cells, on the other hand, the strands were not apparent until a concentration of about 1.5 *M* dextrose was reached (Plate I, *G*). When they did appear they seemed, if anything, thicker than those in the dehardened cells. Consequently the lack of visibility must have been due to a refractive index about identical with that of the surrounding liquid. The obvious conclusion is that the strands of the hardy cells were more highly hydrated than those of the dehardened cells.

The smooth contours of the protoplasts in the lower concentrations indicate a low consistency. In the higher concentrations, the tension on the strands pulled the surface out, making it irregularly dentate; this occurred in a concentration of 3 *M* in hardy cells and in a lower concentration (1.5 *M*) in the case of the dehardened (Plate I, *I* and *C*). The consistency or plasticity of the dehardened protoplasm obviously rises more rapidly on dehydration than does that of the hardy protoplasm. This is to be expected in view of greater dehydration indicated by the more rapid increase in refractive index. In the highest concentrations (2.5–3 *M*) the strands of the dehardened cells became so rigid and inextensible that some of them actually snapped (Plate I, *E*). This never occurred in the hardy cells, even in much higher concentrations such as supersaturated (6 *M*) dextrose (Plate I, *J*).

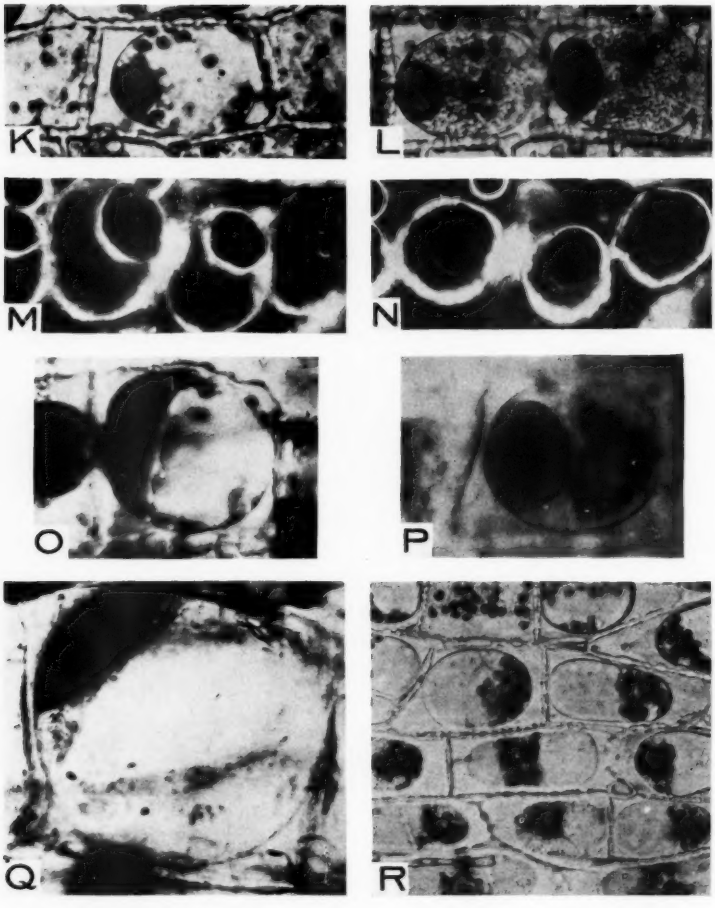
In the lowest concentrations, the strands of the dehardened cells frequently showed spherical regions that appeared to be droplets (Plate I, *B* and *C*). This would indicate a low consistency in these concentrations and would again indicate that near their respective isotonic points, dehardened protoplasm has a lower consistency than hardy protoplasm. However, it is also

PLATE I



A-E. *Cortical section of a dehardened Hydrangea twig in 0.6, 1.0, 1.5, 2.0, and 3.0 M dextrose, respectively. In A, the section is upside-down relative to the others.*

F-J. *Cortical section of a hardy Hydrangea twig in 1.0, 1.5, 2.0, 3.0 M and supersaturated (about 6 M) dextrose. In H, the cell at the left of the other photographs is now in the centre.*



K. *Dehardened cortical cell of Cornus in M dextrose, injected with an oil drop (Nujol).*

L. *Hardy cortical cells of Cornus in a solution of M sodium chloride + calcium chloride (9 : 1), each injected with an oil drop (Nujol).*

M. *Dehardened cortical cells of Cornus in M dextrose, each injected with an oil drop (Nujol).*

N. *The same transferred to about 3 M dextrose.*

O. *Dehardened cortical cell of Cornus injected with an oil drop (Nujol) in M dextrose, transferred to about 2 M, then deplasmolysed.*

P. *Hardy cortical cell of Cornus injected with an oil drop (Nujol) in a solution of M sodium chloride + calcium chloride (9 : 1), transferred to 4 M, then deplasmolysed. (The same result was obtained with dextrose.)*

Q. *Cabbage cell with injected oil drop, stretched by outer cytoplasmic surface (the mesoplasm can be seen to have collected in the angles between the stretched oil drop and the outer cytoplasmic surface.*

R. *Systrophy in dehardened cortical cells of Catalpa after three hours in 4 M dextrose.*

conceivable that these apparent droplets are merely a type of vacuolation (due perhaps to syneresis) which may frequently be seen in protoplasm (9) and which yields no information concerning the consistency of unaltered protoplasm.

Similar tests with *Catalpa* cells yielded similar results, though the strands were finer and less readily observed. Even the dehardened cells, in which the osmotic pressures were very low and which plasmolysed perfectly convexly, showed strand breakage in the higher concentrations. No breaks were observed in hardy cells.

It may be objected that the strands were pulled out to a greater length in the dehardened than in the hardy cells, since the volume reduction of the protoplast was greater, and that this and not a difference in ductility was the cause of the break. But it appears extremely unlikely that such a slight difference in the extent of stretching has any appreciable effect. Strands from free protoplasts have been pulled out to lengths many times the diameter of the protoplast itself without breaking. Furthermore, it is quite probable that the strand length was as great in hardy cells in 6 *M* dextrose as in dehardened cells in 2.5 *M* dextrose, especially since the protoplast contraction in the final stages occurred more at one side than at the ends.

When plasmolysis is concave, the conditions of the strands may be quite different. Sometimes one or two were seen connecting the concave surface to the wall. In the case of dehardened cells, however, focusing on the upper or lower wall revealed a sort of irregular network. This was never observed in hardy cells. Its significance is not clear, though it may be due to torn strands.

From these results it seems evident that the ectoplasm of non-hardy cells is much more readily dehydrated than that of hardy cells. Its refractive index therefore rises more rapidly, the surface of the protoplast is pulled out more easily by tension on the cytoplasmic strands, which eventually become so brittle that they burst in the case of non-hardy but not in hardy cells.

The Shape of Injected Oil Drops

Chambers and Höfler (1) and Chambers and Kopac (2) have shown that an oil drop released near a protoplast snaps onto it. In the following investigation this fact has been used to study the protoplasmic surface of hardy and non-hardy cells.

A micropipette was forced through a pit in the wall of a plasmolysed cell, and thrust close to the surface of the protoplast. The oil was then ejected from the micropipette onto the protoplast surface. In some cases protoplasts freed from their walls were similarly injected, with essentially the same results. A mineral oil (Nujol) with an interfacial tension of 35 to 40 dynes, and olive oil or oleic acid with a tension of 5 to 10 dynes were used. The tensions were measured both in the dextrose and the balanced sodium chloride and calcium chloride (9 : 1) solutions which served as plasmolytes.

Cortical cells of *Cornus* twigs and parenchyma cells of cabbage petioles were investigated. The former were of two types, "vacuolate" and "avacuolate" (7).

The drop established itself instantaneously at the protoplast surface (i.e., it "penetrated"). The interfacial forces molded it into a lens-shaped body that was either equally or unequally bi-convex or even plano-convex. When the curvature was unequal the stronger convexity was toward the inside of the cell, indenting the vacuole quite obviously when the latter was stained with neutral red. There were no apparent differences in shape, whether hardy or non-hardy cells, "vacuolate" or "avacuolate" cells, or even cells from different kinds of plants were compared (Plate II, *K*, *L*).

This behaviour is, of course, true only of small oil drops. With increase in size of the drop, the inner surface changed from convex to plane and finally even to concave if the drop was several times the size of the cell, since surface tension varies with the size of the drop.

Nujol penetrated more easily than olive oil. In fact, only large drops of the latter entered the protoplast at all. When oleic acid penetrated, it dissolved in the protoplasm, producing moribund changes.

In general, ease of penetration of any one oil was similar in different cells plasmolysed to the same degree—even if different kinds of solutions were used (i.e., dextrose or a balanced solution). Strong plasmolysis, using a solution with an osmotic concentration of 100 atm., inhibited the penetration of an oil drop, unless it was several times the size of the contracted protoplast. Thus in the case of dehardened *Cornus* cells plasmolysed in 2 *M* dextrose, the protoplast snapped onto the oil rather than vice versa. Yet even in the strongest solutions, the volume of hardy *Cornus* cells could not be sufficiently reduced to cause this.

A distinct difference between hardy and non-hardy cells was obtained as follows. After injecting an oil drop into a protoplast immersed in a weak plasmolyte (25 to 50 atm.) the section was transferred to a stronger solution (100 atm. or more). Whether the cells were hardy or not, this transformed the oil drop from a lens to a sphere (Plate II, *M*, *N*). If the protoplast was then allowed to deplasmolyse partially, the drop stretched, becoming in some cases flattened and even strongly crescentic. But this reaction depended on the hardiness of the cell as well as on the degree of plasmolysis previous to deplasmolysis. Non-hardy *Cornus* or cabbage cells showed marked flattening if the plasmolyte concentration was 50 atm. or more (Plate II, *O*), hardy cabbage cells if it was 100 atm. or more, whereas hardy *Cornus* cells failed to show any flattening even after plasmolysis in a solution with an osmotic concentration of over 200 atm. (Plate II, *P*). In the latter case there was merely a reversion from the spherical shape (which resulted from the stronger plasmolysis after injection of the oil drop) to the original lens shape. In the non-hardy cells, on the other hand, the sphere stretched to a crescentic layer even before deplasmolysis reached the stage in which the drop

had originally been lens-shaped. If oil drops were injected into other cells of this same tissue, they assumed a practically normal bi-convex or plano-convex shape, in contrast to the flattened shape in the cells into which oil had been injected before further plasmolysis and deplasmolysis.

After strong plasmolysis, if deplasmolysis was allowed to proceed far enough, non-hardy cells, of course, burst. Just prior to this the oil drop reached its maximum degree of extension. This latter phenomenon, in fact, always permitted prediction of the bursting. As a result of the rupture the oil immediately rounded up owing to the release of the tension.

When a sufficiently thick layer of protoplasm was visible, the stretching of the oil drop was seen to depend solely on the surface membrane (Plate II, *Q*). The liquid nature of the protoplasm below this membrane was indicated by the active Brownian movement.

Strong plasmolysis obviously caused solidification or gelation of the membrane of non-hardy but not of hardy protoplasts. It is apparently this solidification that is responsible for the deplasmolysis injury and strand rupture noted above.

Discussion

Two facts are fully established: (1) When in equilibrium with the same dehydrating force, the protoplasm of non-hardy cells has a higher consistency than that of hardy cells. (2) In hardy protoplasts maintained in a strongly contracted condition the ectoplasm does not become rigid, whereas in non-hardy protoplasts contracted for the same time and to a considerably lesser degree, the ectoplasm becomes quite rigid.

These two facts can be explained only on the basis of a greater hydrophily in hardy protoplasm; this increase appears to be more striking in the ectoplasm than in the mesoplasm. Consequently, the membrane of hardy protoplasts is more resistant toward dehydrating forces and less easily stiffened than that of non-hardy protoplasts. It is less easily ruptured on subsequent stretching (whether by deplasmolysis or tension on its strands) and has less of a flattening effect on attached oil drops. Its refractive index does not rise so much on dehydration.

Such an increase in hydrophily of the plasma membrane has already been suggested by Scarth and Levitt (7) as the cause of the increased permeability during hardening. The difference is so great that the mesoplasm itself must also be affected (as, indeed, was found to be the case (7)), even if to a lesser degree. This accounts for the differences in the physical properties of the protoplasm as a whole. Yet at moderate degrees of dehydration, when the protoplasm is still fluid, it may conceivably have a higher consistency in hardy cells.

The two facts listed above are also of vital importance from the point of view of frost resistance. They indicate that at any one freezing temperature, the physical properties of protoplasm are much more markedly altered in non-hardy than in hardy cells. The second one points to an interesting

question. Of what importance is the time factor in frost injury? From analogy with deplasmolysis injury, one would expect it to be of prime importance. This point will be considered in a later paper.

Acknowledgments

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References

1. CHAMBERS, R. and HÖFLER, K. Protoplasma, 12 : 338-355. 1931.
2. CHAMBERS, R. and KOPAC, M. J. J. Cellular Comp. Physiol. 9 : 331-343. 1937.
3. GERM, H. Protoplasma, 18 : 260-280. 1933.
4. GESSNER, F. Ber. deut. botan. Ges. 58 : 2-22. 1940.
5. LEVITT, J. Plant Physiol. 14 : 93-112. 1939.
6. LEVITT, J. and SCARTH, G. W. Can. J. Research, C, 14 : 267-305. 1936.
7. LEVITT, J. and SIMINOVITCH, D. Can. J. Research, C, 18 : 550-561. 1940.
8. SCARTH, G. W. and LEVITT, J. Plant Physiol. 12 : 51-78. 1937.
9. SCARTH, G. W. and LLOYD, F. E. Science, 65 : 599-600. 1927.

EFFECTS OF TALC DUSTS CONTAINING PHYTOHORMONE, NUTRIENT SALTS, AND AN ORGANIC MERCURIAL DISINFECTANT ON THE ROOTING OF DORMANT *TAXUS* CUTTINGS[1]

By N. H. Grace[2] and J. L. Farrar[3]

Abstract

Dormant *Taxus cuspidata* cuttings were treated with talc dusts containing 1- and 2-γ-naphthylbutyric acid at concentrations of 0, 500, 1000, and 2000 p.p.m., each taken separately and in combination with 0, 0.1, 1, and 10% of a mixture of nutrient salts and 0 and 50 p.p.m. of ethyl mercuric bromide. Data on the number of rooted cuttings failed to disclose any significant treatment effects. However, the number and length of roots per rooted cutting were increased by the phytohormone in all concentrations, the effect increasing with ascending concentration. Data for most of the other criteria indicated injurious effects from the phytohormone. The average length of new growth was increased by both the 1 and 10% concentrations of nutrient salt in the presence, but not in the absence, of 1000 p.p.m. of the phytohormone. The 10% concentration of nutrient salts decreased the number of cuttings with new growth and the number of such cuttings that were rooted. Organic mercury treatment failed to have any significant effects.

Previous communications have reported on the effects of treating plant cuttings with phytohormones, cane sugar, and organic mercurial disinfectants incorporated in a talc carrier (6, 7). Beneficial effects were obtained by watering the sand in which Norway spruce cuttings were propagated, with a dilute solution of nutrient salts (5). Beneficial and injurious effects were noted when cuttings were soaked in nutrient solutions in the presence and absence of indolylacetic acid (12, 13). In consequence, it was considered of interest to investigate the effects of nutrient salts in dust mixtures. Since the interaction effects of various chemical treatments are often of importance in propagation (6, 7, 11), the nutrient salts were also used in combination with phytohormones and disinfectants. The present communication describes the results of an experiment in which cuttings of *Taxus cuspidata* Sieb. and Zucc. were treated with talc dusts containing a mixture of isomeric naphthylbutyric acids, nutrient salts, and ethyl mercuric bromide. *T. cuspidata* was selected since it is known to root without great difficulty (1, 14, 16, 17).

Experimental

The experiment involved the use of a factorial series of talc dusts containing naphthylbutyric acid at concentrations of 0, 500, 1000, and 2000 p.p.m. (parts of chemical per million parts of talc mixture by weight), each taken separately and in combination with 0, 0.1, 1, and 10% of a mixture of

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nutrient salts and 0 and 50 p.p.m. of ethyl mercuric bromide (4)*. The naphthylbutyric acid was an isomeric mixture of 1- and 2-γ-naphthylbutyric acids†. This chemical has been shown to have physiological activity of about the same order as naphthylacetic acid (9). The individual members of the mixture of nutrient salts and their relative proportions were the same as those of a previously used nutrient solution (5) which, in turn, was based on Hoagland's nutrient solution (15). Master dusts containing phytohormone, nutrient salts, and organic mercury, each separately, were prepared first. Admixture of these and dilution with ball-milled talc permitted the ready preparation of the entire series of 32 dusts.

There were 10 cuttings to a group and three completely randomized replicates of each treatment, providing one level of precision for comparison of all the main effects and their interactions. In addition to the 32 treatments of the factorial series each replicate contained duplicate groups of 10 untreated cuttings. The entire experiment required 1020 cuttings.

Prepared cuttings‡ of 1939 wood, without a heel and ranging from 6 to 8 in. in length, were treated and planted immediately in a relatively coarse brown sand (10). Although it appears likely that better rooting might have been obtained through the use of a peat-sand mixture (10, 11), sand only was chosen, as nutrient effects from the peat might otherwise have masked the action of the nutrient salts added by the dusts. The cuttings, in groups of 10, were sprayed with water prior to dusting to ensure the adhesion of a substantial quantity of dust, and were then planted at a depth of about 1.5 in. (18), on February 15, 1940. The medium was maintained at a temperature of about 72° F. by electrical bottom heat cables. The room temperature ranged around 65° F., the maximum variation being from 50 to 80° F. For the first week after planting, the propagation frame was covered by a factory cotton shade to reduce light intensity and maintain a high relative humidity. The shade was removed for the remainder of the propagation period. The cuttings were watered heavily at planting and thereafter sprinkled and the sand maintained moist. More recent results with Norway spruce (10) suggest that the watering conditions in this experiment were not optimum. The cuttings were removed for examination May 8, 1940.

Record was made of the number of cuttings with new growth, rooted, with new growth and rooted, callused, and surviving. The number of roots was counted and their lengths were measured. Similarly the number and lengths of all new growth shoots were determined. From these observations were calculated the number and lengths of roots per rooted cutting, the mean root

** The ethyl mercuric bromide used in this experiment was prepared by a method developed in the Chemistry Division, National Research Laboratories, Ottawa, by Dr. A. Cambron. This procedure yielded a product consisting of 80% ethyl mercuric bromide and 20% ethyl mercuric chloride.*

† The mixture of isomeric naphthylbutyric acids was prepared by Dr. R. H. Manske, Division of Chemistry, National Research Laboratories.

‡ Prepared cuttings were supplied by the Federal District Commission, Ottawa, through the kindness of Mr. E. I. Wood.

length, the number and length of new growth shoots per cutting with new growth, and the average length of new growth. All data were analysed by the analysis of variance procedure. Data on numbers of cuttings were subjected to the inverse sine transformation prior to analysis (2).

Results

In Tables I and II are given results of the analyses of variance of the observations. Survival counts were not considered in detail, as very few cuttings with no callus formation survived. For analysis, the number of callused cuttings was combined with the number rooted. In Table I the analyses refer to all the treatments. Table II refers to all treatments except those in which a concentration of 2000 p.p.m. of phytohormone was used, since the meagre data on new growth for this part of the experiment were not suited to the analysis of variance procedure. A number of statistically significant effects from phytohormone and nutrient salt treatment were disclosed by the data. The interactions are not presented in detail in the tables, since, with one exception (Table II), they were all insignificant. Organic mercury failed to have any effect. Data on the significant effects are given in the following tables.

Averaged over the entire experiment, about 27% of the cuttings rooted. About 12% of the untreated cuttings, 17% of the talc treated controls, and 60% of those receiving the best individual treatment were rooted. However,

TABLE I

ANALYSIS OF VARIANCE OF RESPONSES OF *Taxus*. CUTTINGS TREATED WITH DUSTS CONTAINING NAPHTHYLBUTYRIC ACIDS, NUTRIENT SALTS, AND ETHYL MERCURIC BROMIDE

Source of variance	Degrees of freedom	Mean square						
		Number of cuttings		Number of roots per rooted cutting	Length of root per rooted cutting	Mean root length	Number of cuttings with new growth	Number of rooted cuttings with new growth
		Rooted	Rooted and callused					
Replicates	2	77.0	35.6	101.7	7890	0.09	19.8	32.0
Untreated versus all others	1	667.9	28.6	476.7***	47064*	51.53	254.9	294.3
Treatments:								
Phytohormone	3	416.5	3966.3***	1031.6***	66938***	17.15	4888.9***	945.6***
Nutrient salts	3	243.8	251.8	14.9	649	12.24	327.4*	380.6**
Organic mercury	1	234.4	45.4	0.7	38	16.67	21.1	114.8
Interactions	24	102.0	139.8	35.2	6674	18.16	94.4	88.0
Error	67	855.7	123.7	41.1	7168	19.91	94.5	80.3

* *Exceeds mean square error, 5% level of significance.*
** *Exceeds mean square error, 1% level of significance.*
*** *Exceeds mean square error, 0.1% level of significance.*

variability in rooting was so great that no differences could be demonstrated statistically. This result emphasizes the importance of designing such experiments in a manner to permit of some test of the significance of the data.

TABLE II

SUPPLEMENTARY ANALYSIS OF VARIANCE OF RESPONSES OF *Taxus* CUTTINGS

Source of variance	Degrees of freedom	Mean square		
		Number of new growth shoots per cutting with new growth	Length of new growth per cutting with new growth	Average length of new growth shoots
Replicates	2	1.06	1375.2*	25.1
Untreated versus all others	1	0.06	165.9	65.7*
Treatments:				
Phytohormone	2	0.39	550.9	228.8***
Nutrient salts	3	0.35	394.8	13.7
Organic mercury	1	0.61	0.2	14.2
Interactions	17	0.18	325.7	26.9*†
Error	51	0.45	400.2	13.0

* *Exceeds mean square error, 5% level of significance.*
*** *Exceeds mean square error, 0.1% level of significance.*
† *Significance depends entirely on the interaction of phytohormone × nutrient salts.*

TABLE III

AVERAGE RESPONSES OF *Taxus* CUTTINGS ON TREATMENT WITH TALC DUSTS CONTAINING 1- AND 2-γ-NAPHTHYLBUTYRIC ACIDS

Response	Naphthylbutyric acid concentration in talc, p.p.m.				Necessary difference, 5% level
	0	500	1000	2000	
Number of rooted and callused cuttings					
Transformed data	55.3	40.1	35.4	24.3	6.4
Per cent	65.4	42.9	34.6	19.6	
Number of roots per rooted cutting	3.5	9.2	14.8	18.5	3.7
Length of roots per rooted cutting, mm.	35.9	98.4	140.6	153.0	48.8
Number of cuttings with new growth					
Transformed data	51.1	38.5	30.4	17.1	5.6
Per cent	59.6	39.2	26.7	12.1	
Number of rooted cuttings with new growth					
Transformed data	29.8	31.4	28.3	17.5	5.2
Per cent	25.4	28.8	23.3	12.1	
Average length of new growth shoots, mm.	19.0	23.5	24.8	—	2.1

Data on the average effects of phytohormone-nutrient salt treatment are given in Tables III to V. These results demonstrate significant effects from both phytohormone and nutrient salts applied in a carrier dust. The physiological effects of naphthylbutyric acid in all concentrations was brought out most clearly by data on the number and lengths of roots per rooted cutting. The highest concentration, 2000 p.p.m., appeared somewhat too high in some respects, however, as injurious effects were noted, particularly in regard to new growth and percentage of cuttings rooted. Although depression of new growth in *Ribes* cuttings following treatment with the 2000 p.p.m. concentration of indolylbutyric acid has been reported (4), injurious effects from this concentration of the acid on *Taxus*, however, are not in agreement with the findings of other investigators (14).

TABLE IV

AVERAGE RESPONSES OF *Taxus* CUTTINGS ON TREATMENT WITH TALC DUSTS CONTAINING NUTRIENT SALTS

Response	Nutrient salt concentration in talc, %				Necessary difference, 5% level
	0	0.1	1.0	10.0	
Number of cuttings with new growth					
Transformed data	36.4	36.1	35.9	28.8	5.6
Per cent	37.5	37.1	35.8	27.1	
Number of rooted cuttings with new growth					
Transformed data	28.5	28.8	28.9	20.8	5.2
Per cent	25.0	25.4	23.8	15.4	

As was the case in the nutrient watering of Norway spruce cuttings (5), the significant effects of nutrient salt dust treatments related to the new growth (Table IV). It is apparent that 10% is too great a concentration of nutrient salt in the dust, as its effect, on the whole, was injurious. However, in conjunction with the 1000 p.p.m. concentration of phytohormone, even at this level there resulted a substantial increase in the average length of new growth shoots. It may be concluded that there are conditions under which the

TABLE V

INTERACTION EFFECTS OF PHYTOHORMONE AND NUTRIENT SALT TREATMENT ON THE AVERAGE LENGTH OF NEW GROWTH OF *Taxus* CUTTINGS, MM.

Phytohormone concentration in talc, p.p.m.	Nutrient salt concentrations in talc, %			
	0	0.1	1.0	10.0
0	19.5	19.3	19.2	17.9
500	25.5	22.3	21.5	24.9
1000	21.9	22.2	28.5	26.8

Necessary difference, 5% level, 3.0.

combination of nutrient salts and phytohormone has beneficial effects in vegetative propagation.

Previous communications have indicated that data on the number and length of roots often provide more information as to the effect of treatment than counts of the number of cuttings rooted (3, 8). The results of this experiment emphasize the importance of this conclusion and also indicate that quantitative data on new growth are of value in indicating physiological activity following treatments.

References

1. CHADWICK, L. C. Cornell Agr. Expt. Sta. Bull. 571. 1933.
2. COCHRAN, W. G. Empire J. Exptl. Agr. 6 : 157-175. 1938.
3. GRACE, N. H. Can. J. Research, C, 17 : 247-255. 1939.
4. GRACE, N. H. Can. J. Research, C, 17 : 305-311. 1939.
5. GRACE, N. H. Can. J. Research, C, 17 : 312-316. 1939.
6. GRACE, N. H. Can. J. Research, C, 17 : 321-333. 1939.
7. GRACE, N. H. Can. J. Research, C, 18 : 13-17. 1940.
8. GRACE, N. H. Can. J. Research, C, 18 : 283-288. 1940.
9. GRACE, N. H. Can. J. Research, C, 18 : 457-468. 1940.
10. GRACE, N. H. and FARRAR, J. L. Can. J. Research, C, 18 : 591-598. 1940.
11. GRACE, N. H., FARRAR, J. L., and HOPKINS, J. W. Can. J. Research, C, 18 : 566-577. 1940.
12. GRACE, N. H. and THISTLE, M. W. Can. J. Research, C, 17 : 317-320. 1939.
13. GRACE, N. H. and THISTLE, M. W. Can. J. Research, C, 18 : 122-128. 1940.
14. HITCHCOCK, A. E. and ZIMMERMAN, P. W. Contrib. Boyce Thompson Inst. 10 : 461-480. 1939.
15. HOAGLAND, D. R. Science, 52 : 562-564. 1920.
16. HUBERT, B., RAPPAPORT, J., and BEKE, A. Mededeelingen Landbouwhoogeschool Opzoekingsstations Gent, 7 : 291-360. 1939.
17. OLIVER, R. W. Sci. Agr. 18 : 379-387. 1938.
18. STOUTEMYER, V. T. Proc. Am. Soc. Hort. Sci. 36 : 817-822. 1938.

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A STUDY OF THE MALE GENITALIA IN CALYPTRATE DIPTERA, BASED ON THE GENUS *GONIA* MEIGEN (DIPTERA: TACHINIDAE)[1]

BY FRANK O. MORRISON[2]

Abstract

A review of the major papers on the 'genitalia' of calyptrate Diptera indicates the need of establishing the evident homologies within the group, studying homologies with other forms, studying the rotation of the parts known to occur in some forms, and studying the musculature.

Gonia has 'sternite V' of the abdomen, and all succeeding segments, modified for effecting copulation. The 'posterior angles of sternite V' are produced. A small apparent 'tergite VI' may be secondarily cut off from the previously fused segments VI and VII. A narrow 'tergite VII' articulates on the left side with an asymmetric 'sternite VI and VII'. Segment VIII has disappeared. 'Tergite IX', the genital tergite, is large. 'Sternite IX', a shovel-shaped ventral sclerite, is anterior to the 'phallus'. Segment X is represented by the 'lateral lobes of sternite X', the 'tenth sternal plate', and the 'anal forceps' and, in related forms, (not in *Gonia*), by the 'parapodial plates'.

The 'phallus' consists of a 'phallobase' with 'dorsal spine', a bell-shaped 'aedeagus', and protrusible 'penis'. The 'aedeagus' is formed of a dorsal 'paraphallus', lateral 'hypophalli', membranous 'ventralia', and apical membranous 'praeputium'. Anterior and posterior pairs of 'basal phallic appendages' surround the 'phallobase'. Internally a 'double apodeme' extends forward.

The term 'genitalia' is used to refer to the entire modified apex of the abdomen.

Introduction

The similarity in the structure of the apex of the abdomen, among higher Diptera, has long been recognized. However, the dissimilarity in this structure, from that among lower Diptera, or from that among other orders of insects, is none the less striking. For these reasons there has been no lack of studies on dipterous 'genitalia' but little agreement as to the segmental origin and general homologies of the parts.

An apparently varying number of apical abdominal segments have been modified for the purposes of effecting copulation. To this modified abdominal apex many terms have been applied. Thus Westhoff (87), Snodgrass (79), Feüerborn (43), Petzold (71), and others refer to this apical portion of the abdomen as the "hypopygium". Metcalf (58) refers to it as "the post-

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abdomen". Patton (68) and Gibbins (45) argue that the term "terminalia" should be used and "genitalia" reserved for segment IX and its appendages; yet the term "genitalia" has been widely used in the literature to refer to the entire structure in question. The present writer has adopted the term 'genitalia' to refer to the entire apex of the abdomen and all associated structures.

Other morphological terms used in this paper are for the most part those employed by Snodgrass (81) or listed by La Torre-Bueno (54). Throughout the text single quotation marks enclose nomenclature employed by the writer. Table I lists the terms used by those writers whose works were reviewed. It is not always possible to be certain of the exact synonomy of such terms. Other writers have given similar comparative tables. In some cases these do not agree with the one presented here. This is due to differences in interpretation and possibly to mechanical errors in building tables. Thus Awati (27) lists his "alar projections of the theca" as synonomous with the "alar projections" of Hewitt (47), the "double apodeme" of Wesché (85), and the "great apodeme" of Lowne (55). An examination of these papers reveals that the "alar projections" of Hewitt and Awati are the 'posterior angles of sternite IX', which are directed posteriorly, whereas the "double apodeme" of Wesché and "great apodeme" of Lowne is the 'apodeme of the phallus' and is directed anteriorly. It is not perfectly clear from Awati's diagrams whether this is his "median process" or not. Again Awati appears to have misinterpreted Lowne with regard to segment VI, listing Lowne's segment VI? as equal to his segment V, while Lowne definitely states that sternite V is the last visible sternite and is deeply emarginate behind, thus identifying it with Awati's sternite V.

Similarly Petzold (71) appears to have confused Brüel's (31) terms "lamina inferior" and "lamina superior" and Lowne's terms "hypophallus" and "paraphallus", wrongly synonomizing the first of the former with the second of the latter.

The appended list of references gives some idea of the extent of the accumulated literature on the copulatory apparatus or genitalia of the order Diptera alone. A review of even the major papers on the genitalia of calypyrate Diptera must in the interests of brevity be omitted from this publication; however, at least an indication of the varying interpretations by the different authors may be found in Table I. The numbers for papers cited in the list of references and those used for figures continue on from those appearing in an earlier paper by the writer on the taxonomy of *Gonia**.

The Male Genitalia of *Gonia*

General Considerations

It has not been possible, during the preparation of this paper, to investigate the matter of the rotation of the apical abdominal segments as described by Feüerborn (43) and Schräder (78). Keuchenius (52) indicates the ejacula-

* *Can. J. Research, D, 18 : 336-362. 1940.*

TABLE I

COMPARISON OF TERMS USED BY DIFFERENT WRITERS

—	Writer	(1)	(2)	(3)	(4)	(5)
1	Terms used in this paper	Sternite V Lobes of sternite V	Tergite VI	Sternite VI and VII	Tergite VII	Tergite IX
2	Lowne, (55)	Sternite V	Tergite VI	Epipleuron	Tergite VII	Tergite VIII Progenital tergum
3	Brüel, (31)	Sternite V	Tergite VI	Chitinring?	Tergite VII	Tergite VIII
4	Hewitt, (47)	Sternite V Primary forceps	Tergite VI	Sternite VI	Tergite VII	Tergite VIII
5	Wesché, (85)	Laminae superiores	—	—	—	—
6	Berlese, (28)	Sternite VI Primo forcipe	Tergite VIII	Sternite IX	Tergite IX	Tergite X
7	Newstead, (63)	Sternite VI	Tergite VII	—	Tergite VIII	—
8	Awati, (27)	Sternite V Accessory forceps	Tergite VI	Sternite VII	Tergite VIII	Tergite IX Genital tergite
9	Newell, (62)	Sternite V	Tergite VI and VII	Sternite VI and VII	Tergite VIII	Tergite IX
10	Müeller, (60, 61)	Sternite V	Tergite VI	—	—	Tergite VIII
11	Feüerborn, (43)	Sternite VI	Tergite VII	Halbring	Tergite VIII	Tergite IX
12	Petzold, (71)	Sternite V	Tergite VI	Sternite VI	Tergite VII	Tergite VIII
13	Schräder, (78)	Sternite VI	Tergite VII	Basalring Extension of tergite 7 and 8	Tergite VIII	Tergite IX
14	Cole, (33)	Sternite V	Tergite VI	?	Tergite VII and VIII	Tergite IX
15	Patton, (70)	Sternite V Lateral processes	Tergite VI	Sternite VI	Tergite VII	Tergite X
16	Townsend, (82)	Sternite V Anterior forceps	Tergite VI	Sternite VII	Tergite VII	Tergite IX
17	Snodgrass, (81)	Sternite V	Tergite VII	Sternite VIII	Tergite VIII	Tergite IX

TABLE I—*Continued*

COMPARISON OF TERMS USED BY DIFFERENT WRITERS—*Continued*

—	Writer	(6)	(7)	(8)	(9)	(10)
1	Terms used in this paper	Sternite IX	Tergite X Parapodial plates	Sternite X	Lobes of sternite X	Anal cerci (segment X)
2	Lowne, (55)	Progenital sternum	?	Sternite IX	Valvae externae	Valvae internae
3	Brüel, (31)	Sternite VIII	Tergite IX	—	Valvula lateralis	Valvula medialis
4	Hewitt, (47)	Body of the penis	—	—	Sternite VII	Sternite VIII
5	Wesché, (85)	Part of the theca	—	—	Forceps inferior	Forceps superior
6	Berlese, (28)	—	Tergite XI	—	Forceps secundus Sternite X	Mesocerci Tergite X
7	Newstead, (63)	—	—	—	Editum	Superior claspers
8	Awati, (27)	Vinculum Body of the penis	—	—	Inner claspers	Anal cerci Segment X
9	Newell, (62)	Sternite X ?	—	—	Tergite X	Mesocerci Tergite X
10	Müeller, (60, 61)	—	—	—	Seitenlappen ?	Forceps
11	Feüerborn, (43)	Sternite VII	Tergite X	—	Gonopöden	Cerci
12	Petzold, (71)	Vordere Gabelplätte Sternite 7 and 8	Tergite IX	Hintere Gabelplätte	Valvula lateralis	Valvula medialis
13	Schräder, (78)	Gabelplätte Sternite VII	Tergite X	—	Valvula lateralis	Valvula medialis
14	Cole, (33)	Sternite IX ?	?	—	Surstyli Lobes of tergite IX	Cerci
15	Patton, (70)	Ninth tergo-sternum	—	—	Ninth coxites	Anal cerci
16	Townsend, (82)	Vinculum Sternite IX	Tergite X	—	Lobes of Sternite X	Paraprocts or anal cerci Segment XI
17	Snodgrass, (81)	Sternite IX	Tergite X	—	Lobes of Tergite IX	Lobes of Tergite X

TABLE I—*Continued*

COMPARISON OF TERMS USED BY DIFFERENT WRITERS—*Continued*

—	Writer	(11)	(12)	(13)	(14)	(15)
1	Terms used in this paper	Posterior angles of sternite IX	Anterior basal phallic appendages	Posterior basal phallic appendages	Anterior angles of tergite IX	Processus longus
2	Lowne, (55)	Posterior angles	Anterior gonapophyses	Posterior gonapophyses	?	Epipleural ridge
3	Brüel, (31)	Arm der Winkelhëbels	Hakenfortsätze	Paramere	Gelenkfortsätze	Processus longus
4	Hewitt, (47)	Alar processes of the penis	—	—	—	—
5	Wesché, (85)	—	Palpus genitalium	Forceps interior	—	—
6	Berlese, (28)	—	Corpus penes	Ala corporis penis	—	—
7	Newstead, (63)	—	—	Inferior claspers?	—	—
8	Awati, (27)	Alar projections	Appendages of the vinculum? Lateral processes	Lateral processes?	—	—
9	Newell, (62)	—	—	Appendages of segment VIII	—	Appendages of segment XI?
10	Müeller, (60, 61)	—	—	—	Gelenkfortsätze	Processus longus
11	Feüerborn, (43)	—	—	—	—	—
12	Petzold, (71)	—	Häkenfortsätze	Paramere	Processus brevis	Processus longus
13	Schräder, (78)	Hebelfortsätze	Häkenfortsätze	Paramere	Gelenkfortsätze der VIII T.	Processus longus
14	Cole, (33)	—	Genital palpi	Interior forceps	—	—
15	Patton, (70)	Lateral processes to coxites	Anterior part of paramere	Posterior part of paramere	Processes of tergite X	—
16	Townsend, (82)	Vincular apophyses	Anterior gonapophyses	Posterior gonapophyses	—	—
17	Snodgrass, (81)	Arms of Sternite IX	Median plates?	Free pair of lobes embracing aedeagal base	—	Lateral bars to segment X ?

TABLE I—*Continued*

COMPARISON OF TERMS USED BY DIFFERENT WRITERS—*Continued*

—	Writer	(16)	(17)	(18)	(19)	(20)	(21)
1	Terms used in this paper	Phallus	Phallic base	Spine	Aedeagus	Paraphallus	Hypophallus
2	Lowne, (55)	Penis	Bulb	Azygos spine	—	Paraphallus	Hypophallus and ventralia
3	Brüel, (31)	Penis	Pars basalis	Dorn	—	Lamina superior	Lamina inferior
4	Hewitt, (47)	Penis	Theca?	Superior apophysis	—	—	—
5	Wesché, (85)	—	Theca?	Spinus titillatorius		Paraphallus	Hypophallus
6	Berlese, (28)	—	—	Apophysis spinosa penis	—	—	—
7	Newstead, (63)	—	—	—	—	—	—
8	Awati, (27)	Theca	Theca	—	—	—	—
9	Newell, (62)	—	—	—	—	Appendages of segment IX	End or horns
10	Müeller, (60, 61)	Penis	Gründglied	Dorn	Mittelstuck	Furca	Appendages of segment X Vomer
11	Feüerborn, (43)	Penis	Theka	Dorn	—	—	—
12	Petzold, (71)	Penis	—	Dorn	—	—	—
13	Schräder, (78)	Penis	—	—	—	Lamina superior	Lamina inferior
14	Cole, (33)	Aedeagus	—	Spinus titillatorius	—	Paraphallus	Hypophallus
15	Patton, (70)	Phallosome	—	Posterior process	Body of phallosome	—	—
16	Townsend, (82)	Aedeagus	Phallotheca	Inferior apophysis of the phallobase	Phallus	Paraphallus	Hypophallus
17	Snodgrass, (81)	Phallus	Phallobase	?	Aedeagus	—	—

TABLE I—*Concluded*

COMPARISON OF TERMS USED BY DIFFERENT WRITERS—*Concluded*

—	Writer	(22)	(23)	(24)	(25)	(26)
1	Terms used in this paper	Ventralia	Praeputium	Penis	Double apodeme	Sperm pump
2	Lowne, (55)	Included in the hypophallus	?	—	Giant apodemes	—
3	Brüel, (31)	Lamina lateralis	Distaler Rand der weichen Innenröhre	—	Tragplätte Sternite IX	Samenpritzt
4	Hewitt, (47)	—	Glans ?	—	—	Ejaculatory apodeme
5	Wesché, (85)	Hypophallus	—	—	Double apodemes	Ejaculatory apodeme
6	Berlese, (28)	—	—	—	—	—
7	Newstead, (63)	—	—	—	—	—
8	Awati, (27)	—	—	—	—	—
9	Newell, (62)	—	—	—	Ejaculatory duct ?	—
10	Müeller, (60, 61)	Seitenmembrane	Endstuck	—	—	—
11	Feüerborn. (43)	—	—	—	—	Sternite IX
12	Petzold, (71)	—	—	—	Penisstütze	Samenpumpe
13	Schräder, (78)	Lamina lateralis	—	—	Tragplätte Sternite VIII	Sternite IX
14	Cole, (33)	—	—	Penis	Double apodeme	—
15	Patton, (70)	—	—	—	Apodeme of the phallobase	—
16	Townsend, (82)	Ventralia	Praeputium	—	Vincular apodeme	—
17	Snodgrass, (81)	—	—	Penis	Apodeme	Ejaculatory bulb

tory duct in *Dexia canina* Fabr. as crossing over the alimentary canal but, though he studied several forms, he does not make a point of the winding of the duct about the canal. Tullock (83) says of *Stomoxys* that the duct "does not encircle the rectum as in *Glossina*." Minchin (59) found it encircling the canal in *Glossina*. It is thus not possible to conclude that the condition is general in Diptera and it is hoped when fresh material is available to investigate this matter in the species concerned here.

Neither has it been possible to do any detailed study of the musculature of the genitalia of *Gonia*. From what limited observations could be made on dried specimens, it appeared that the musculature closely resembled that found by Schräder (78) in *Calliphora erythrocephala*. No muscular connections between 'sternite IX' or the 'apodeme' and tergites V or VI were observed.

It has been possible, however, to thoroughly investigate the external morphology of the genitalia in *Gonia* and to compare it directly with that in many other forms.

Structure and Homologies of the Parts

Gross Structure

The 'genitalia' of *Gonia* consist of the sternite of segment V and the following segments VI to X inclusive. Segment XI has entirely disappeared or is included in X. Segment VI is mostly membranous and folded under segment V. Sometimes, however, a narrow dorsal plate, 'tergite VI' is present behind and beneath 'tergite V'. Ventrally, 'sternites VI and VII' are probably included in an asymmetric sclerite (*S. VI + VII*, Figs. 35, 36), just above the fifth sternite and closely united to it basally. On the left side this combined sternite articulates with 'tergite VII', which is a distinct narrow, dorsal, transverse plate, at rest partly hidden under 'tergite IX'. In the extended position a membranous area intervenes between segments VII and IX. It would seem that segment VIII has disappeared, a condition postulated by Awati (27) and Patton (68). Segment IX is the genital segment and is represented by a large tergite (*T. IX*, Fig. 35) and sternite (*S. IX*, Fig. 35) behind which the 'phallus' is borne. The anus is a long slit-like opening on a membranous area just posterior to 'tergite IX'. No sclerotic 'parapodial plates', such as occur lateral to the anus in some Tachinids (71) are present in *Gonia*. A pair of terminal 'anal forceps' (*A.C.*, Figs. 35, 36, 37) are fused into one structure. Lobes of sternite X (*Lb. S.X.*, Figs. 35, 36, 37) are present. Placed laterally, just anterior to the 'anal forceps' they articulate with the 'forceps' and with 'tergite IX', and by means of long sclerotic rods, 'processi longi' (*proc. l.*) with the 'tenth sternal plate' (*S. pl. X.*).

A pair of 'anterior basal phallic appendages' (*Ant. b. ph. app.*, Fig. 38) are fused to the ninth sternite and appear as outgrowths of it. Posterior to these is a second pair of appendages, the 'posterior basal phallic appendages' (*Post. b. ph. app.*). These are movably articulated. The phallus (*Ph. l.*, Fig. 37) is supported by 'sternite IX' between the extended 'posterior angles' of which it protrudes. Basally it articulates with the large internal 'double apodeme'

(*Ap.*) which extends anteriorly into the body. The 'phallus' itself consists of a phallobase (*Phlb.*), aedeagus (*aed.*), and internal 'penis'.

'Sternite V' (Fig. 36)

STRUCTURE OF THE PARTS

The fifth sternite in *Gonia* is the last externally visible one. It is considerably wider than the preceding sternites. Its lateral edges have folded over ventrally and the posterior lateral corners extended to form two prominent projections, 'the lobes of the fifth sternite' (*Lb. S. V.*, Fig. 36). The inner edges of the external lobes are folded back and, in the 'fissiforceps' group, curiously "escalloped". In all species the inner edges of the lobes bear many heavy black bristles. It is homologous with sternite V or sternite VI of other authors, according to the number of abdominal segments they recognized. The lobes have been variously termed "accessory forceps", "primary forceps", etc. (Table I).

'Tergite VI' (Fig. 35)

The sixth tergite in *Gonia breviforceps* Tothill is represented by a wide membranous area posterior to 'tergite V'. Normally, when the 'genitalia' are in the resting position, this area is invaginated and only exposed narrowly on the sides, where the spiracles are present. The latter structures, then, just show anterior to 'tergite VII'. In some species a short, narrow, dorsal, sclerotized area has been observed. At rest it is half hidden beneath 'tergite VII'. A larger remnant of this tergite is present in *Cynomya cadaverina* (Fig. 32). A portion of 'tergite VI,' in this case, seems to be still adhering to the anterior edge of 'tergite VII', especially at the left end where the combined tergite articulates with 'sternite VI and VII'. In *Cynomya* these sclerites also articulate at their right ends. 'Tergite VI' is well developed in *Calliphora* (Fig. 31).

In *Drosophila melanogaster* segments six and seven have evidently fused completely. The segment posterior to five, and exactly similar to it in the structure of the tergite, has two spiracles on each side in the connective membrane just below it. A similar condition has been described by Awati (27) in *Lispa* where he considered it a further step in the evolution of the 'genitalia'.

Since in *Gonia* and other related forms the penultimate spiracle lies just on the edge of the apparent seventh tergite and the ultimate within it and, since in such forms as *Musca domestica* Linn. (Fig. 30), the apparent sixth and seventh tergites remain completely fused on the right side and overlap on the left where the apparent seventh crosses the sixth to articulate with 'sternite VI and VII', it would appear that these two tergites, once fused into one plate, became reduced, and secondarily separated in the interests of flexibility at that point. If this is the case their exact limits, i.e., whether or not the split came on the old line of fusion, is not certain and would explain the position of the spiracles and the apparent connection of what seems to be sternite VI with what seems to be tergite VII. As pointed out above, in *Cynomya cadaverina* Desv. there is evidence that this division is not along the old line of fusion as a portion of the original sixth tergite appears to be

united with seven along its anterior border and to form a lobe at the left end. This lobe is involved in the articulation with 'sternite VI and VII' which takes place on the suture.

'Sternite VI and VII' (S. VI + VII, Figs. 35, 36)

Immediately above the fifth sternite and connected closely to its base is an asymmetric sclerite which ends free in a somewhat expanded lobe in the membrane on the right side of the fly, but continues up on the left side to articulate with the seventh tergite. It appears to be homologous with a similar sclerite in other calyptrate flies (Figs. 30, 32).

The close association of this sclerite with the seventh tergite has led some investigators (27) to list it as sternite VII. This interpretation assumes that sternite VI has disappeared. Snodgrass (81) considers that the entire sixth segment has disappeared. The apparent seventh tergite then becomes the eighth and this asymmetric sternal plate, closely associated with it, sternite VIII. Still other writers questioned the sternal nature of this sclerite. Lowne (55) called it an "epipleurite" while Schräder (78) interpreted it as a prolongation of his tergites VII and VIII. The pupal study by the last named author threw little light on the origin of this plate though he observed it early in the development and determined that it took no part in the rotation of the genitalia.

Comparative morphology at the present time does not seem sufficient to finally settle this matter. However, if the complete union of segments VI and VII in *Drosophila*, and in *Lispa* (27) is noted, it may be seen how such a compound structure as 'sternite VI and VII' might develop and maintain connection with a secondarily separated apparent 'tergite VII' and still remain closely united to 'sternite V.' In *Cynomya* (Fig. 32) this asymmetric ventral sclerite maintains its articulation with the tergal plates on both sides though that on the left side is stronger and resembles the articulation in other forms. As previously pointed out, the detached sclerite labelled 'tergite VI' seems to be only a portion of this tergite. The rest remains attached to 'tergite VII'. Possibly it is all one sclerite which breaks easily when handled. Certainly the anterior lobe of 'tergite VII' on the left side seems to be a part of 'tergite VI' and it is just at the union of this lobe and 'tergite VII' that the asymmetric sternal plate articulates. For these reasons the sternite in question is here considered as 'sternite VI and VII'.

'Tergite VII' (Fig. 35)

The seventh tergite in *Gonia* is a narrow strip of sclerotized chitin, bounded anteriorly by connective membrane and normally lying almost against 'tergite V'. If a narrow apparent 'tergite VI' is present the two may be fused on the right side. Posteriorly 'tergite VII' is bounded and often overlapped by the large genital tergite, 'tergite IX'. A spiracle is present near each end. At the right end 'tergite VII' is free in the connective membrane or fused with the similarly free end of 'tergite VI'. The left extremity widens and forks,

one branch ending free and one articulating with the extremity of 'sternite VI and VII' just posterior to 'spiracle VI'.

In *Musca* (Fig. 30) the structure is similar, the left end being enlarged and crossing over the end of 'tergite VI' to meet 'sternite VI and VII', while the right ends of 'tergites VI and VII' are fused. In *Calliphora* (Fig. 31) 'tergite VII' is a prominent sclerite and according to Schräder is bound to the anterior end of 'sternite IX' by a strong muscle band. As previously noted this segment is fused with segment VI in *Drosophila* and *Lispa*.

Segment VIII

Segment VIII in *Gonia* has disappeared or is entirely membranous. There is no indication of the presence of any eighth pair of spiracles. Reichardt (77) found the sclerites of segment VIII very narrow and no spiracles present in some Asilids such as *Laphria flava* (Fig. 24). In this species also the hypopygium including segment VIII has rotated through 180 degrees necessitating a wide connective membrane in this area. In *Tabanus* (Fig. 26) segment VIII is also somewhat reduced. Again in the Dolichopodidae, Snodgrass (79) notes the tendency of segment VIII to lose its spiracles and to almost entirely disappear on the right side (Figs. 25, 25*a*). In the Syrphidae, according to Metcalf (58), segment VIII is large but possesses no spiracles and is represented by the tergite only, which is often asymmetrical (Figs. 27, 27*a*). In *Drosophila* (Fig. 29), there is a wide connective membrane and great flexibility between segments VII and IX, but no spiracles or sclerites. Because of this tendency of segment VIII to weaken and lose its spiracles and because of the wide connective membrane in this area in calyptrate flies, the assumptions of Awati (27), Patton (68), and Townsend (82), that segment VIII has disappeared seem more logical than the view of Snodgrass (81) that segment VI is missing or that of earlier workers that the anterior abdominal segment is missing. The view that the genital segment is VIII, which has lost its spiracles, seems untenable in the light of the known reasonably constant occurrence of the opening of the male duct behind 'sternite IX' in insects.

Petzold (71) noted the presence of a clear, transverse line across 'sternite IX' (his "Gabelplätte") and held it as evidence of the presence of two sternites, VII and VIII, in this structure (Fig. 34*a*). The line is evident in the homologous ventral plate in *Gonia* and in other forms examined. If it is indicative of the double origin of the plate, sternites VIII and IX must be involved as the exit duct is posterior to segment IX. However, in *Tabanus* (Fig. 26) that portion of 'sternite IX' which is beneath 'sternite VIII' is very similarly differentiated and has been designated the "apodeme of sternite IX" by Newell (62).

Schräder (78) found 'sternite IX' bound to 'tergites VIII and IX' by muscular connections while Brüel (31) found muscles connecting the same plate and the 'apodeme' (his "Tragplätte") to 'tergites V and VI'. All in all, the evidence is conflicting and seems too slight to postulate the presence of 'sternite VIII' in the structure here called 'sternite IX' or in any other structure observed.

'Tergite IX' (Figs. 35, 36)

The ninth or genital tergite is a large, dorsally convex plate with a V-shaped emargination in its posterior edge. It is folded under laterally and its 'antero-lateral angles' extend as sclerotized rods (*a.a.T. IX*) which articulate with the outside of the extended 'posterior angles of sternite IX' (*Post. a.S. IX*) and are closely bound to these by connective membranes. The posterior angles articulate with processes on the 'anal forceps'. The sides are emarginate posteriorly, and from beneath them extend the 'lobes of sternite X', which articulate with them. Ventrally, connective membrane extends across between the folded lateral edges from the 'ninth sternite' posteriorly to the 'anal forceps'. In the connective membrane covering the V-shaped posterior emargination is found the long slit-like anal opening. The close connection of this sclerite with 'sternite IX', its position in relation to the anus, and its apparent similarity in form and function to 'tergite IX' in the Syrphids (Figs. 27, 27*a*), to 'tergite IX' in the Dolichopodidae (Fig. 25), seem to determine which sclerite it is. It seems improbable that it is tergite VIII (71), or tergite X, as suggested by Townsend (82).

'Sternite IX' (Figs. 35, 35)

'Sternite IX' is a slightly arcuate, shovel-shaped sclerite. Anteriorly it is rounded. The 'posterior' "corners" or 'angles' are produced caudad and upward into processes on either side of the 'phallus'. In *Gonia* these produced 'posterior angles of sternite IX' articulate laterally with the 'anterior angles of tergite IX' and apically with 'sternite X', the sternal plate posterior to the 'phallus'. In forms in which 'sternite X' is absent or fused to these arms (Fig. 33), they appear to articulate apically with the 'processi longi' of the 'lobes of sternite X'.

In *Gonia* (Fig. 38) the posterior portion of 'sternite IX' on either side of the 'phallus' is prolonged into two ventrally directed lobes, the 'anterior basal phallic lobes'. These are hollow, slightly curved, sickle-shaped, sclerotic projections bearing hairs on their apices and with their upper posterior angles prolonged to articulate with the phallic base. Petzold (71) found comparable lobes ("Häkenfortsätze") in *Ernestia rudis* but considered them as distinctly separate from 'sternite IX' (his "Gabelplätte"). He shows them articulating with two projections on 'sternite IX' and through a pair of very small sclerites at their bases with the 'phallus' and the 'anterior basal phallic lobes'. Whether these 'posterior basal phallic lobes' are a part of 'sternite IX', true appendages (62), or part of the 'phallus', is uncertain. They are present in many calyptrate forms but rudimentary in the housefly and absent in *Drosophila*. No homologous structures appear to be present in lower forms.

Distinctly articulated posteriorly with the above mentioned 'anterior basal phallic lobes' are a second pair of elongate, almost straight, tubular, sclerotic lobes, the 'posterior basal phallic lobes' (Fig. 38). Snodgrass (81) considers these structures in *Pollenia rudis* as phallic lobes rather than as true appendages. He states that they are not musculated. Schräder (78) found that

they develop in the pupa from the second pair of genital lobes. This is of interest in view of the work done in tracing the development of genital apendages through immature forms in other insects. This work has been well summarized by Snodgrass (81) as follows:

"Studies on the development of the male genitalia in Trichoptera, Lepidoptera, and Hymenoptera have shown that the tubular phallic organ of these insects is formed during larval development by the union of a pair of genital lobes that grow out at the sides of the gonapore [Zander, (88, 89, 90); Singh Pruthi, (73, 74), Mehta (57)]. It is possible, therefore, that these larval phallic lobes of higher insects are homologues of the lateral phallomeres of Mantidae and Blattidae. According to Zander, the primitive phallic lobes divide each into a median lobe and a lateral lobe, the two median lobes uniting to form the intromittent organ, while, in Trichoptera and Lepidoptera, the lateral lobes move to the sides and become articulated to the margins of the annulus of the ninth segment. We might, therefore, regard the median lobes as gonapophyses of the gonapods, and the lateral lobes (valvae or harpagones) as the styli. However, since it is claimed by Mehta that the lateral lobes in Lepidoptera arise separately from the median lobes, we cannot accept it as established that the gonapods of the male insect take any part in the formation of the intromittent organ, though there appears to be little doubt that they give rise to the styli or the movable claspers of the genital segment."

If Schräder's observations are accurate it would seem that these 'posterior basal phallic appendages' are the true appendages of segment IX and hence homologous with the large claspers of lower Diptera (Tipulidae, etc.), Trichoptera, and Lepidoptera (55). Certainly there do not seem to be any other structures present in calyptrate Diptera that represent the appendages of segment nine. Awati (27) and others have considered the parts here called lobes of 'sternite X' as the true appendages or at least as the coxites of segment nine. The evidence against this interpretation lies in the dorsal position, and dorsal and post-phallic articulation of the structures. Awati himself has called attention to this difficulty and solved it by merely stating that in his Syrphid type the appendages of the genital segment are dorsal and not ventral as in *Bibio*. Snodgrass considers that the appendages of segment IX are missing and that the structures in question, the 'posterior basal phallic appendages' are secondary outgrowths of the connective membrane about the base of the 'phallus'. He thus terms them phallic rather than 'periphallic' structures and his view is being adopted for the present.

Tergite X

'Parapodial plates'

There are no sclerites in the connective membrane surrounding the anus in *Gonia*. In *Drosophila* (Fig. 28) two small oval sclerites occur, posterior and lateral to the anus. These are here termed the 'parapodial' plates. In *Cynomya* (Fig. 32) they are represented by small triangular plates, on each

side of the anus, and in *Calliphora* (Fig. 31) by narrow lateral plates termed "tergite IX" by Brüel. Petzold (71) shows similar plates in some tachinids, in some cases free, in others fused with 'tergite IX'.

'Anal forceps' (Figs. 35, 36, 37)

The 'anal forceps' are the terminal structures in *Gonia*, when the genitalia are extended. At rest, however, they are folded beneath the abdomen with their apices in the genital pouch above the 'lobes of sternite V'. They are formed of two lateral sclerites, closely fastened together along the mid-dorsal line and divaricating only slightly at the apex. The lateral edges of the plates are rolled under and joined, at least basally, with connective membrane which is continuous with that extending ventrally across 'tergite IX'. Dorsally the forceps articulate with 'tergite IX' and laterally with the 'lobes of sternite X'. They vary greatly in width, depth, curvature, and setal vestiture, according to the species.

The anal forceps of *Pollenia rudis* and *Calliphora* are completely separate lateral lobes. In *Musca* (Fig. 30) they are flat and plate-like, but closely united with a very narrow sclerite inserted between them. Lowne (55) labelled them "sternite X". In *Cynomya* (Fig. 32) they are rudimentary, the small setae-bearing areas posterior to the 'parapodial plates' being the only indication of them. Similarly reduced 'anal forceps' are shown by Petzold (71) in certain Tachinidae. *Drosophila* (Figs. 28, 29) has two curved strips of slightly sclerotized chitin, below the parapodial plates, attached to the 'lobes of sternite X' and marked by strong setae on their edges. These appear to represent the 'anal forceps'. They are evident as posterior lobes of segment X in the Syrphids (Fig. 27*a*) and appear in some forms to be jointed. The folded under parts of 'tergite X' in *Tabanus* may represent these structures. The Dolichopodidae and Asilidae show no very similar structures but it seems probable that they are contained in the plates of the anus-bearing proctiger.

Snodgrass (81) and earlier workers find the anal forceps bound to 'tergite IX' by muscular connections which closely resemble the dorsal, intersegmental muscle bands.

'Lobes of sternite X' (Figs. 35, 36)

In *Gonia* there are present a pair of lateral lobes anterior to the 'anal forceps'. These are peculiarly contorted sclerites. Ventro-laterally, on each side of 'tergite IX' and somewhat beneath it, lies a somewhat triangular sclerite with the apex of the triangle curved ventrally and almost meeting its fellow from the other side in the membranous floor of the genital chamber. The base of the triangle is directed dorsally. Anteriorly it projects beneath 'tergite IX' and articulates with it, while posteriorly it articulates with the 'anal forceps'. Extending ventro-laterally from the anterior side of the triangle is a hollow, tubular, finger-like sclerite. The base of this projection is emarginate anteriorly. The projection is usually supplied with numerous setae apically. From the inside of the base a process, the 'processus longus', extends anteriorly in the floor of the genital chamber to articulate with the 'tenth sternal plate'

or directly with the posterior angles of 'sternite IX'. The origin of these curious triangular sclerites with their lobes and processes forms one of the major problems in the homologizing of the structures and explaining their segmental nature.

In *Tabanus* (Fig. 26) the tergite and sternite of segment X are conspicuous. Each is divided into lateral halves. The segment, the anus-bearing proctiger, extends anteriorly to sternum IX. The ventral plates of segment X articulate anteriorly with the 'ninth sternite', posterior to the phallic base.

In the Syrphidae (Fig. 27*a*) there are two styli-like lobes which articulate with 'tergite X' and 'sternite X'. *Drosophila* (Figs. 28, 29) has two large lobes extending on either side, outside of, but connected with, the 'anal forceps'. These lobes have definite 'processi longi' which connect them with the phallic base, posteriorly. In *Ernestia rudis* (Fig. 34) and in *Calliphora* the lobes resemble those in *Gonia* but are more strongly developed. *Schräder* (78) finds the 'processus longus' connected by muscles to 'tergite IX' but Snodgrass (81) considers these as unmusculated lobes. The 'lobes of sternite X' in *Cynomya* (Figs. 32, 33) have moved back to the place generally occupied by the 'anal forceps' and have united basally. The 'processi longi' have broadened and fused to form a definite 'tenth sternal plate' posterior to the 'phallus' and very similar to that present in some Syrphids. In *Musca* the lobes are flattened ventral plates mistaken for the seventh sternite by Hewitt (47).

The position of these lobes and their articulation, always posterior to the 'phallus', indicates their origin from the tenth segment and probably from the sternite of the same. They have frequently been termed the coxites of segment nine but their position relative to the 'phallus' makes this interpretation doubtful. Their shape, articulation, and lack of direct musculation suggests an origin other than from true appendages.

In view of the divided tenth sternite of *Tabanus*, the position of the lobes in question in *Gonia* and other calyptrate flies, and the formation of a ventral plate posterior to the 'phallus' by the fusion of the 'processi longi' in *Cynomya*, it is tentatively held here that these lobes have their origin in the tenth segment and are probably sternal. This is the view held by Townsend (82) though he states no reasons for it.

'Tenth sternal plate' (Figs. 33, 34, 36)

Posterior to the 'phallus' in *Gonia* (Fig.36) lies a narrow, transverse, ventral plate with which the anterior ends of the 'processi longi' articulate. In *Pollenia rudis* this plate is turned on edge extending into the genital cavity and forming with the 'posterior angles of sternite IX' a basal shield for the 'phallus'. In *Musca* (Fig. 30) such a plate is absent or has split and the parts fused with the 'posterior angles of sternite IX'. The 'tenth sternal plate' in *Cynomya* has probably fused with the flattened and fused 'processi longi'. *Ernestia rudis* (Fig. 34) shows a condition similar to that in *Gonia*. Petzold (71) has designated this structure the "hintere Gabelplätte". Lowne (55) mentions a sternal plate posterior to the 'phallus' in *Calliphora* but does not name it.

In the Syrphidae (Fig. 27*a*) the 'tenth sternal plate' curiously resembles the composite plate in *Cynomya*. Such a plate, posterior to the 'phallus', might arise from a fusion of the extended 'posterior angles of sternite IX'. However, it seems more probable that it represents 'sternite X'.

The 'Phallus and Phallic Structures' (Fig. 38)

The 'anterior and posterior basal phallic appendages' have been dealt with previously. (See under 'Sternite IX'). There remains the 'phallus' proper and its 'double apodeme'.

The 'Phallus'

The theory of the segmental origin of the '*phallus*' in Diptera or any insect has been almost completely discarded. There is some evidence, according to Schräder (78) of its origin in Diptera from portions of appendages which have fused. However, as pointed out in the quotation from Snodgrass (see under 'Sternite IX') such an origin has not been proved the case in any insect. There is, however, a general basic plan according to which this structure in most calyptrate Diptera may be interpreted.

In general the 'phallus' is an unpaired median structure. It is frequently referred to as the 'penis', but is a composite structure of more than the 'penis' proper which it ensheathes. Townsend (82) refers to it as the "aedeagus". Lowne (55) suggested a terminology. Brüel (31) gave a more complete terminology. Müeller (60) took *Lucilia caesar* as a basis and developed a new set of terms for the structure in a "basic plan". Townsend (82) has chosen from earlier workers the names he prefers. Since the morphological homologies with structures in other insects are very doubtful as yet, any of these sets of terms serve the purpose of description equally well. The terms chosen for use here are those most frequently seen. Apparent synonomies have been listed in Table I, but since one term may in some cases cover parts of structures separately named by another worker exact synonomy is not always indicated.

In *Gonia* the 'phallobase' is tubular. Its anterior end articulates basally with the 'double apodeme' and is produced dorsally to articulate on each side with dorsal extensions of the 'anterior basal phallic appendages'. A membranous area occurs latero-dorsally in the 'phallobase'. Anteriorly it is produced into a dorsal 'spine'.

Closely attached to the 'phallobase' at right angles and with lateral apodemes extending into it, is the 'aedeagus'. It is narrow basally and expanded apically. A slightly sclerotized plate begins as the apodemes within the 'phallobase' and extends on the dorsal side of the 'aedeagus' where it divides forming a dorsal, spine-like structure, the 'paraphallus' or united 'paraphalli' ("Furca" of Müeller), and two large lateral expansions which envelop the greater part of the 'aedeagus' laterally and may be narrowly united basally on the ventral surface, the 'hypophalli' ("Vomer" of Müeller). These structures are sclerotized in successive small, scale-like patches, giving the surface a chequered appearance under high magnification.

The ventral part of the aedeagus, the 'ventralia', is entirely membranous. The apical 'praeputium' is likewise membranous. The penis or true intromittent organ is a long narrow tubular structure equal in length to the aedeagus and concealed within it when at rest. This structure was observed once while material was being treated in cold potassium hydroxide but was so delicate that it was destroyed before a drawing could be prepared.

The 'Double Apodeme'

Extending upward and anteriorly from the 'phallobase' into the body, above 'sternite IX', is a sclerotic apodeme. Basally, where it articulates with the 'phallobase', this structure is narrow. It extends into segment V and broadens out transversely fan-like, with a vertical keel-shaped projection below. Many muscles are attached to this apodeme and these govern the movements of the phallus.

Lowne (55) termed the similar structure in *Calliphora* the "great apodemes". Other writers have homologized it with certain sternites (Table I) while Wesché (85) uses the terms "double rod", "apodeme of the penis", and "double apodemes". The double nature of this structure is evident in some forms but in most calyptrate Diptera there is one fused structure. Newell (62) has shown *Tabanus* to have two entirely separate and distinct apodemes. Their homology with the structure in question is, however, uncertain. In *Musca* apodemes are entirely lacking and in *Drosophila* seem to be represented by a sclerotized portion of the ejaculatory duct. It does not seem that we are yet justified in postulating an origin, sternal or otherwise, for this structure.

As pointed out in the introduction, Awati (27) misinterpreted certain early authors in their uses of the terms for the "double apodeme".

References

27. Awati, P. R. Indian J. Med. Research, 3 : 510-529. 1916.
28. Berlese, A. Rev. Patalog. vegetable, 9 : 345-357. (Cited by Berlese, 29). 1902.
29. Berlese, A. Gli insetti. 1. Societa Editrice, Libraria, Milano. 1909.
30. Böttcher, G. Deut. entomol. Z. 525-544. 1913.
31. Brüel, L. Zool. Jahrb. Anat. 10 : 511-618. 1897.
32. Christophers, S. R. Indian J. Med. Research, 3 : 371-394. 1915.
33. Cole, F. R. Proc. Calif. Acad. Sci. 16 (4) : 397-499. 1927.
34. Crampton, G. C. Psyche, 25 : 47-51. 1918.
35. Crampton, G. C. Bull. Brooklyn Entomol. Soc. 13 : 49-68. 1918.
36. Crampton, G. C. Can. Entomol. 52 : 178-183. 1920.
37. Crampton, G. C. Psyche, 27 : 34-41. 1920.
38. Crampton, G. C. Can. Entomol. 53 : 72. 1921.
39. Crampton, G. C. Trans. Am. Entomol. Soc. 48 : 207-225. 1923.
40. DeCoursey, R. M. Ann. Entomol. Soc. Am. 20: 368-384. 1927.
41. Edwards, F. W. Ann. Trop. Med. 14 (1) : 23-40. 1920.
42. Escherich, K. Verhandl. Zool.-bot. Ges. 42 : 225-232. 1893.
43. Feüerborn, H. J. Zool. Anz. 55 : 189-212. 1922.
44. Freeborn, S. B. Am. J. Hyg. 4 : 188-202. 1924.
45. Gibbins, F. G. Ann. Trop. Med. 29 : 317-327. 1935.
46. Haase, E. Morphol. Jahrb. 5 (15) : 331-435. 1890.
47. Hewitt, C. G. Quart. J. Micr. Sci. n.s. 51 : 395-448. 1907; 52 : 495-545. 1908.

48. HEYMONS, R. Morphol. Jahrb. 24 : 178-204. 1896.
49. HEYMONS, R. Biol. Zentr. 16 : 854-864. 1896.
50. HEYMONS, R. Zool. Zentr. 6 (16): 1-16. 1899.
51. HOLDAWAY, F. G. Bull. Entomol. Research, 24 : 549-560. 1933.
52. KEUCHENIUS, P. E. Z. wiss. Zool. 105 : 501-536. 1913.
53. KLAPALEK, F. Zool. Anz. 27 : 449-453. 1904.
54. LA TORRE-BUENO, J. R. de. A glossary of entomology. Science Press Printing Co., Lancaster, Penna. 1937.
55. LOWNE, B. T. The anatomy, physiology, morphology, and development of the Blow-fly, *Calliphora erythrocephala*. R. H. Porter, Princess Street, Cavendish Square, West London. 1893–1895.
56. MARTINI, E. Arch. Naturgesch. Abt. A, Heft 1, 88 : 134-142. (Cited by Feüerborn, 43). 1922.
57. MEHTA, D. R. Quart. J. Micr. Sci. 76 : 35-61. 1933.
58. METCALF, C. L. Ann. Entomol. Soc. Am. 14 : 169-227. 1921.
59. MINCHIN, E. A. Proc. Roy. Soc. (London). Ser. B, 76 : 531-548. 1905.
60. MÜELLER, A. Arch. Naturgesch. Abt. A, 88 : 45-167. 1922.
61. MÜELLER, A. Verhandl. Zool.-bot. Ges. 73 : 51-111. 1923.
62. NEWELL, N. G. Ann. Entomol. Soc. Am. 11 : 109-142. 1918.
63. NEWSTEAD, R. Bull. Entomol. Research, 2 : 9-36. 1911.
64. NEWSTEAD, R. Bull. Entomol. Research, 2 : 47-78. 1911.
65. NUSBAUM, J. Zool. Anz. 5 : 637-643. 1882.
66. PACKARD, A. S. Proc. Boston Soc. Nat. Hist. 11 : 393-399. 1868.
67. PALMEN, J. A. Morphol. Jahrb. 9 : 169-176. 1884.
68. PATTON, W. S. Ann. Trop. Med. 26 : 347-405. 1932.
69. PATTON, W. S. Ann. Trop. Med. 29 : 363-381 (also 19-33; 73-91; 199-231; 486-496; 517). 1935.
70. PATTON, W. S. and CUSHING, E. C. Ann. Trop. Med. 28 : 107-121; 123-130; 205-216; 217-223; 305-314. 1934.
71. PETZOLD, W. Z. Naturw. 63 : 1-50. 1927.
72. PEYTOUREAU, S. A. Une contribution a l'étude de la morphologie de l'armiture genitale des insectes. Bordeaux. 1895.
73. PRUTHI, H. S. Proc. Zool. Soc. London : 857-868. (Cited by Snodgrass, 81). 1924.
74. PRUTHI, H. S. Trans. Entomol. Soc. London : 127-267. (Cited by Snodgrass, 80). 1925.
75. PRUTHI, H. S. Entomol. Monthly Mag. London, 65 : 198-201. 1929.
76. REES, J. VAN. Zool. Jahrb. 3. (Cited by Schräder, 78). 1889.
77. REICHARDT, H. Z. wiss. Zool. Leipzig. 135 : 257-301. 1929.
78. SCHRÄDER, T. Z. Morphol. Ökol. Tiere, Berlin. 8 : 1-44. 1927.
79. SNODGRASS, R. E. Proc. Calif. Acad. Sci. 3 (3) : 273-294. 1904.
80. SNODGRASS, R. E. Trans. Am. Entomol. Soc. 30 : 179-236. 1904.
81. SNODGRASS, R. E. Principles of insect morphology. McGraw-Hill Book Co., New York and London. 1935.
82. TOWNSEND, C. H. T. Manual of myiology. I, Charles Townsend et Filhos, Itaquaquecetuba, Saõ Paulo, Brazil.
83. TULLOCK, F. Proc. Roy. Soc. (London). 78 : 523-529. 1906.
84. WESCHÉ, W. J. Quekett Micr. Club. 9 (2) : 233-238. 1905.
85. WESCHÉ, W. Trans. Linnean Soc. London. (Zool.) 339-386. 1906.
86. WESCHÉ, W. Trans. Entomol. Soc. London, 283-305. 1908.
87. WESTHOFF, F. Inaugural Dissertation, Munster. 62 pp. (Cited by Snodgrass, 79). 1882.
88. ZANDER, E. Z. wiss. Zool. 67 : 461-489. (Cited by Snodgrass, 81). 1900.
89. ZANDER, E. Z. wiss. Zool. 70 : 190-235. (Cited by Snodgrass, 81). 1901.
90. ZANDER, E. Z. wiss. Zool. 74 : 557-615. (Cited by Snodgrass, 81, and Schräder, 78). 1903.

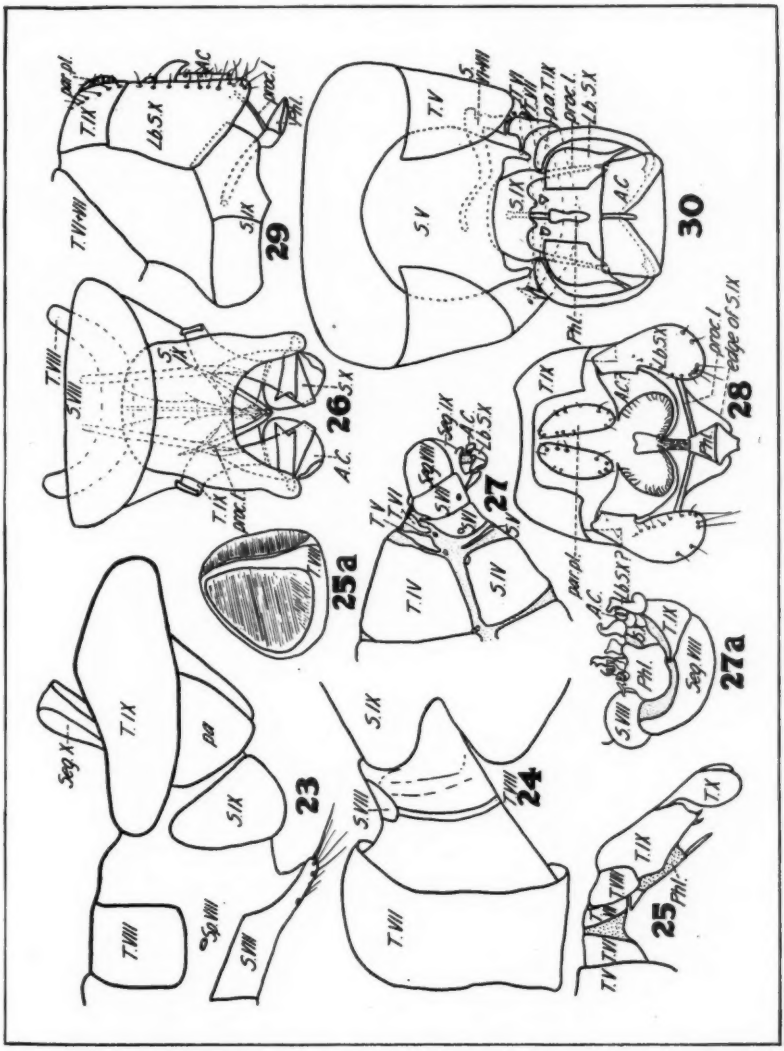

FIG. 23. *Lateral view of the apex of the abdomen of Machimus atricalpus (Asilidae) (After Reichardt, 77).*

FIG. 24. *Lateral view of the apex of the abdomen of Laphria flava (Asilidae) (After Reichardt, 77). Note: hypopygium has revolved through 180 degrees; segment 8 is narrow.*

FIG. 25. *Lateral view of the apex of the abdomen of a Dolichopodid (After Snodgrass, 79).*

FIG. 25a. *The same as 25: segment 8 from the right hand side.*

FIG. 26. *Ventral view of the apex of the abdomen of Tabanus (Tabanidae).*

FIG. 27. *Tip of the abdomen of Eristalis (Syrphidae) (After Metcalf, 58). Numbering of the segments and labelling of the structures by the present writer.*

FIG. 27a. *Diagrammatic representation of the various parts represented in the male genitalia of the Syrphidae, dextro-cephalic view (After Metcalf, 58). Numbering and labelling by the present writer.*

FIG. 28. *Apex of the abdomen of Drosophila melanogaster, viewed from behind.*

FIG. 29. *The same as 28, lateral view.*

FIG. 30. *Ventral view of the apex of the abdomen of Musca domestica.*

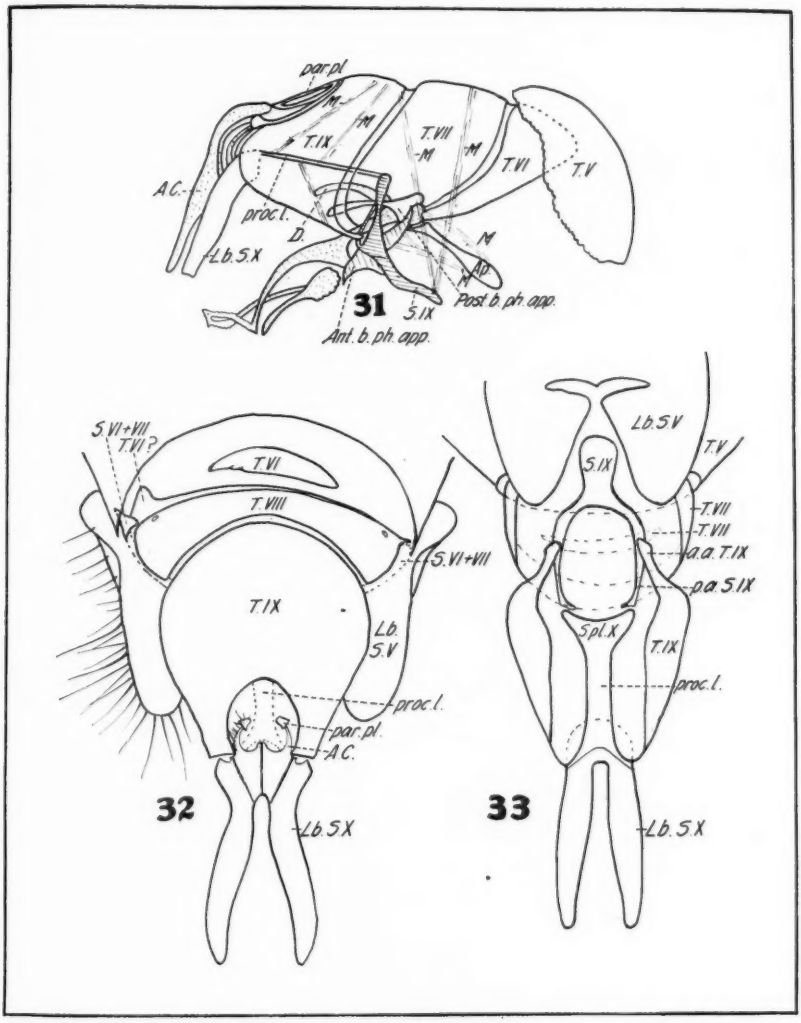

FIG. 31. *Lateral diagram of the apex of the abdomen of Calliphora erythrocephala (After Schräder, 78). Numbering and labelling by the present writer.*

FIG. 32. *Dorsal view of the apex of the abdomen of Cynomya cadaverina Desv.*

FIG. 33. *The same as 32, ventral view, showing the fused, plate-like "processi longi". The 'phallus' and 'phallic' structures have been omitted.*

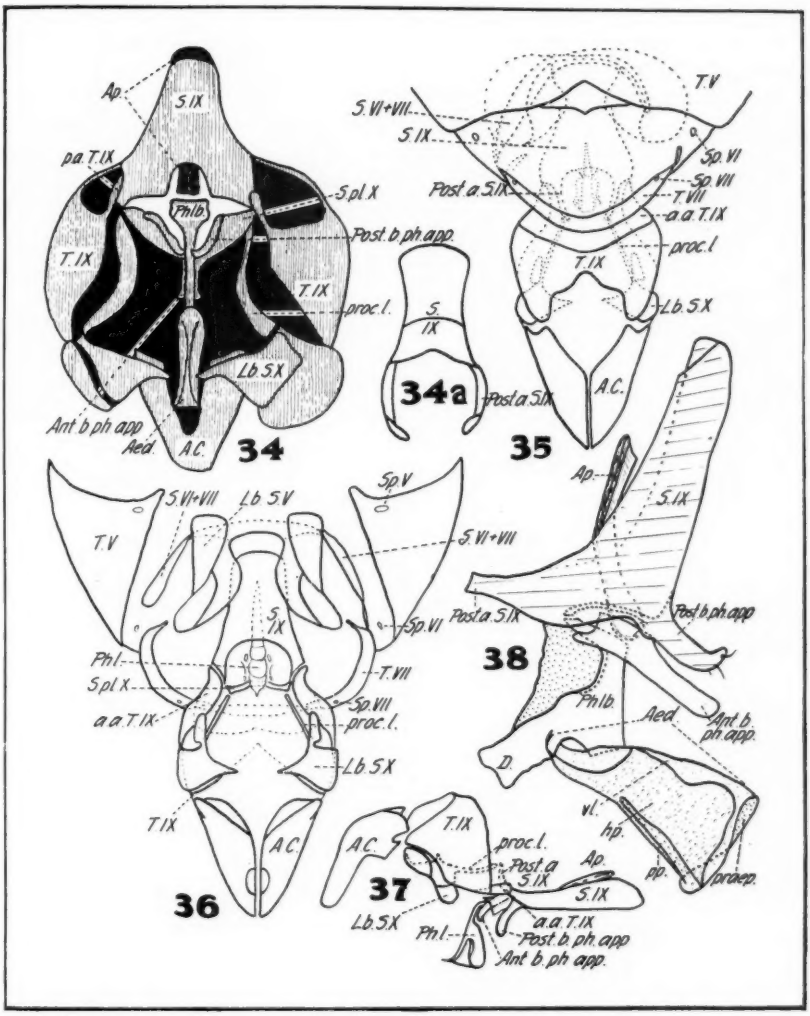

FIG. 34. *Ventral view of the apex of the abdomen of Ernestia rudis (After Petzold, 71). Numbering and labelling by the present writer.*

FIG. 34*a*. *Ventral view of 'sternite IX' of Ernestia rudis (after Petzold, 71).*

FIG. 35. *Dorsal view of the apex of the abdomen of Gonia breviforceps Tothill.*

FIG. 36. *The same as 35, ventral view.*

FIG. 37. *The same as 35, lateral view.*

FIG. 38. *Lateral view of 'sternite IX' and the 'phallic' structures of Gonia fissiforceps Tothill.*

CANADIAN WILTSHIRE BACON

XIV. SEASONAL VARIATIONS IN COLOUR AND COLOUR STABILITY[1]

By C. A. Winkler,[2] W. H. Cook,[3] E. A. Rooke,[4] and A. E. Chadderton[4]

Abstract

Measurements of the colour and colour stability of Wiltshire bacon, cured in a factory known to produce a generally satisfactory product, showed that there were small but significant differences in these properties from time to time, but there was no definite evidence to indicate that these differences could be attributed to systematic seasonal effects.

Introduction

Colour and colour stability are two important attributes of quality in bacon. Previous investigations (3, 4) on this subject have shown that, although Wiltshire sides cured at the same time in the same factory, may differ significantly in colour quality, the main source of variation generally lies in curing sides in different factories. This suggests that the colour quality of bacon depends primarily on the handling and curing practices followed in different establishments. There is also the possibility that colour quality may show seasonal variations, due either to the influence of environment on the animal prior to slaughter or to the effect of minor variations in the handling or curing conditions at certain stages where all the conditions are not subject to close control. The observations of certain practical operators suggested the existence of such seasonal variation in colour. The present study was undertaken to examine this possibility.

Material and Method

All the samples were obtained from a plant known to adhere closely to a practice that produced sides of consistently satisfactory colour quality. One side was taken at random from each weekly batch over a period of 20 months. After maturing at 32 to 35° F. for two weeks after removal from the curing tank, a portion, always taken from the same position in the prime back, was examined in the pale or unsmoked state.

The colour measurements were made with the colour comparators described in earlier publications. The first instrument (1) was used for the early measurements, and the improved form (2) during the remainder of the period. Owing to the higher "dark" constant of the first instrument, the readings so obtained were greater than those observed with the improved

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Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa, Canada. Issued as paper No. 54 of the Canadian Committee on Storage and Transport of Food and N.R.C. No. 959.

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model. For this reason the results with the two instruments must be considered separately.

Estimates of colour stability were obtained from the change in colour during exposure for 24 hr. at 10° C. and 60 to 70% relative humidity. This relative humidity is lower than that employed in earlier measurements, and was chosen to include the effect of both drying and oxidation, comparable with that occurring in retail stores.

It has been shown in earlier papers (3, 4) that there is some variation between the colour and colour stability of different sides cured in the same tank at the same time. This inherent difference between sides must be considered in estimating the significance of differences that might otherwise be attributed to seasonal or time effects. Since it was impossible to provide samples from two sides, each week, it was assumed that the difference observed over a two-week period (three observations: beginning, middle, and end of period) would unlikely be seasonal, and consequently could be taken to represent the difference between sides. The observed differences were therefore analysed into portions attributable to within and between these two-weekly periods, taken as representing the difference between sides and seasonal effects respectively.

Results

The results of these analyses of variance appear in Table I. They show that on the average the differences between two-week periods, indicative of time effects, were usually significant for both the initial colour and colour stability measurements made with both instruments.

Figs. 1 and 2 were constructed from the results of the colour and colour stability measurements in an attempt to determine whether the significant differences, demonstrated between two-week periods, could be attributed to seasonal effects. Each point represents the mean of the three observations taken during each period. The central horizontal line indicates the general mean for all measurements made with the given instrument. The cross-hatched section on each side of the general mean indicates the necessary difference, computed from the variance within periods, between the individual points and the general mean.

It is evident from Fig. 1 that the initial colour during a given period seldom exceeded the necessary difference. The measurements made during June, 1938, with the original instrument were generally significantly below average, and increased to somewhat above average during August, 1938. Subsequent variations did not attain significance. Since the below- and above-average values observed in 1938 did not appear in 1939 when measurements were made with the new comparator there is no evidence to indicate that the difference between periods are attributable to systematic seasonal effects.

Fig. 2 shows the results of the colour stability measurements. The amount of light scattered by bacon generally decreases during exposure, and this was particularly true in these tests conducted under conditions that allowed

TABLE I

RESULTS OF ANALYSES OF VARIANCE ON COLOUR AND COLOUR STABILITY MEASUREMENTS

Source of variance	Degrees freedom	Mean square			Total brightness
		Red	Green	Blue	
Initial colour of internal surface (original instrument)					
Between two-week periods	17	40.28**	36.30**	35.68**	293.05**
Within two-week periods (Difference between sides)	36	8.29	5.24	2.84	42.40
Initial colour of internal surface (new instrument)					
Between periods	9	19.50*	6.90	4.04*	81.02*
Within periods	20	7.34	2.90	1.59	30.86
Colour stability (original instrument)					
Between periods	17	20.51**	3.89**	18.56**	99.94**
Within periods	36	3.84	1.02	2.58	15.32
Colour stability (new instrument)					
Between periods	9	3.48*	1.70**	0.69*	14.97**
Within periods	20	1.20	0.46	0.26	3.75

**Indicates 5% level of significance.*
***Indicates 1% level of significance.*

some drying to occur. In consequence, the initial value minus the final value is positive. This difference is used as the ordinate in the figure. Since the points are all greater than zero some darkening is indicated for all samples. A marked darkening, or instability, occurred during August, 1938, when the colours of the original samples were brighter than average. Otherwise none of the points show a significant departure from the average of all observations made with the original instrument. During August, 1939, the bacon, measured with the new instrument, showed greater stability than the average. This appears to be associated with the darker samples (Fig. 1) obtained at that time. From these results it appears that, although colour stability may be associated with the initial colour of the samples, there is no evidence of definite seasonal trends.

Discussion

Attempts to relate the significant departures from average colour quality to the use of frozen carcasses and to other minor changes in curing practice indicated that these were not the causative factors. Likewise the temperature records for the years 1938 and 1939 failed to show anything that would explain the variations. The significant departures from the average, therefore, remain unexplained.

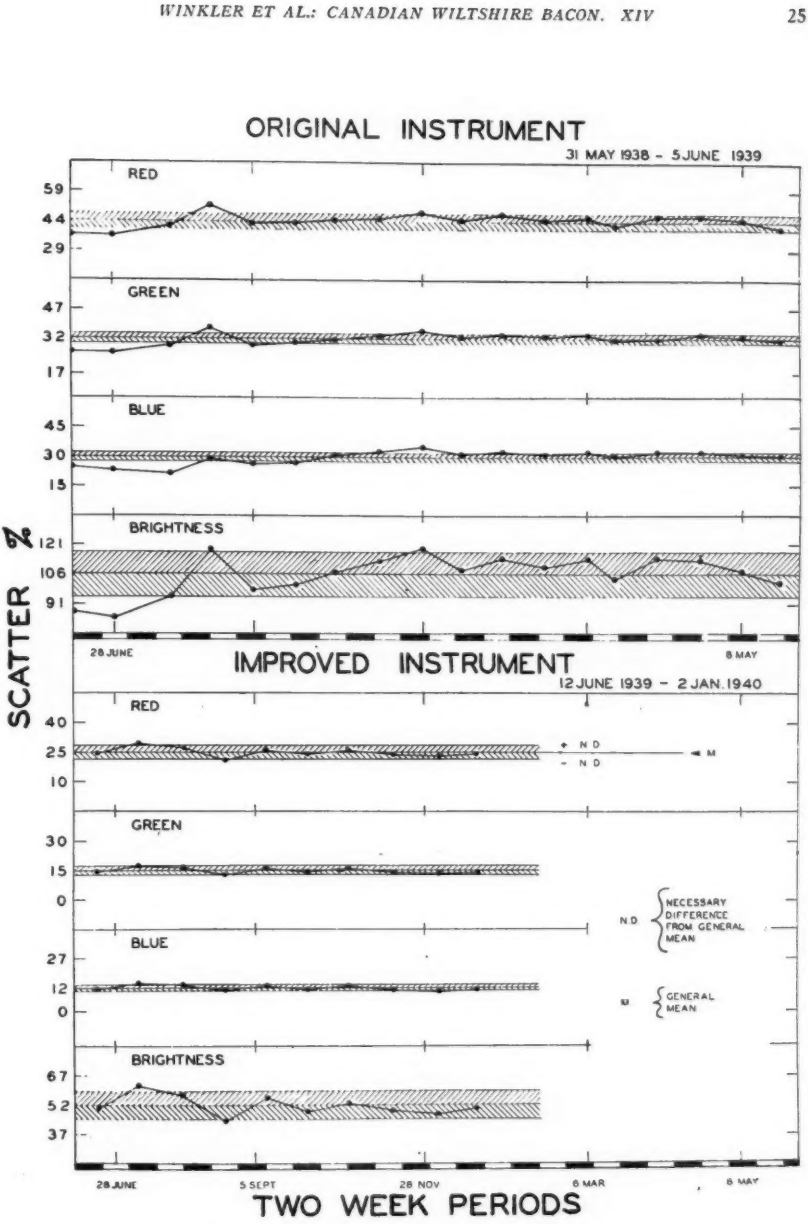

FIG. 1. *Seasonal variations in colour scatter.*

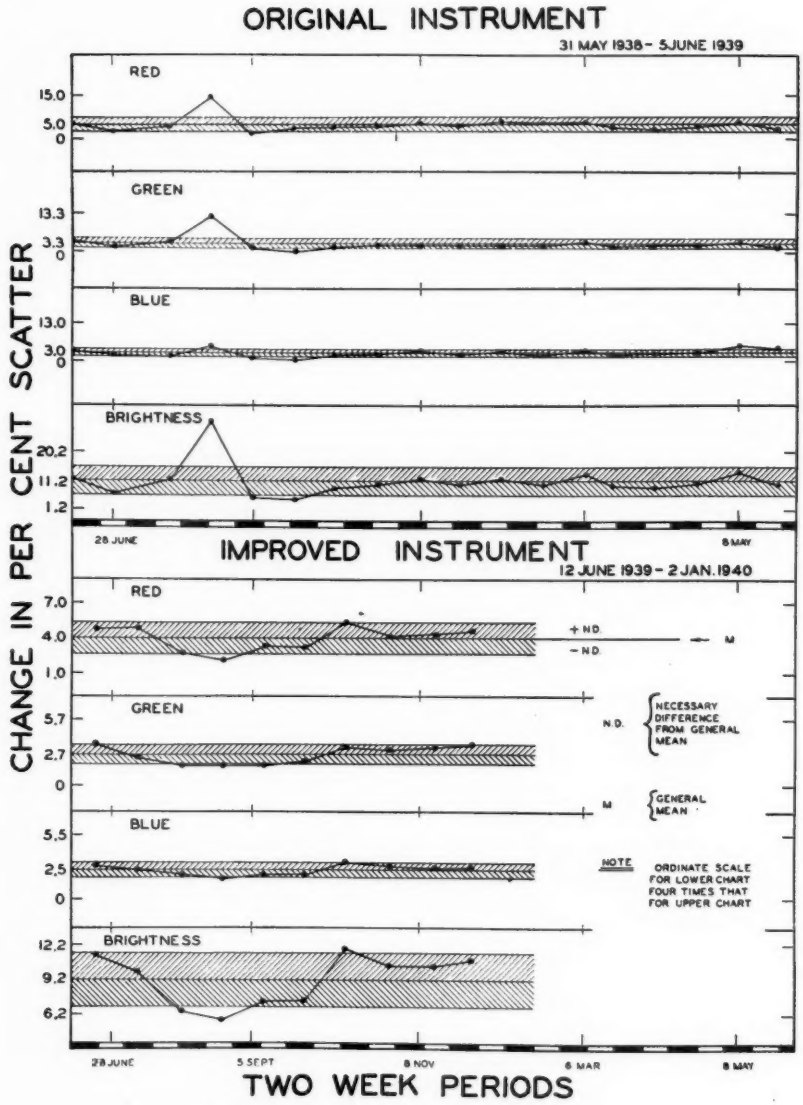

FIG. 2. *Seasonal variations in colour stability.*

It is entirely probable that the curing and handling practices followed in certain factories may enhance possible seasonal variations in colour quality. The results of the present investigation, however, indicate that practices could be adopted that would render the product relatively independent of these effects, but the nature of the detrimental practices, if any, must await the results of future investigations.

References

1. WINKLER C. A. Can. J. Research, D, 17 : 1-7. 1939.
2. WINKLER, C. A., COOK, W .H., and ROOKE, E. A. Can. J. Research, D, 18, 435-441. 1940.
3. WINKLER, C. A., HOPKINS, J. W., and ROOKE, E. A. Can. J. Research, D, 18 : 225-232. 1940.
4. WINKLER, C. A., HOPKINS, J. W., and THISTLE, M. W. Can. J. Research, D, 18 : 217-224. 1940.

A CRITICAL STUDY OF STAFFORD'S REPORT ON "TREMATODES OF CANADIAN FISHES" BASED ON HIS TREMATODE COLLECTION[1]

By Max J. Miller[2]

Abstract

The internal trematodes from fish in the late Dr. Stafford's slide collection have been restudied and described. *Gasterostomum armatum* of Stafford is identified with *Prosorhynchus squamatus* Ohdner, 1905, and *Crepidostomum laureatum* with *C. cooperi* Hopkins, 1932. *Homalometron pallidum* Stafford, 1904, is redescribed and it is suggested that the genus *Homalometron* Stafford, 1904, is synonymous with *Lepocreadium* Stossich, 1904. *Neophasis pusilla* Stafford, 1904, and *Stenakron vetustum* Stafford, 1904, are redescribed and assigned to the family *Allocreadiidae*. *Hemiurus appendiculatus* of Stafford, is demonstrated to represent *H. levinseni* Ohdner, 1905, and *Brachyphallus crenatus* Rudolphi, 1802, while *Fellodistomum incisum* of Stafford represents the species *F. fellis* Olsson, 1868, and *F. agnotum* Nicoll, 1909. Species of the genera *Azygia* and *Otodistomum* are reidentified. *Protenteron diaphanum* Stafford, 1904, is redescribed and the species is referred to the genus *Cryptogonimus* Osborn, 1903, the new combination being *Cryptogonimus diaphanus*.

Introduction

The late Dr. Stafford's report (19) on the "Trematodes of Canadian Fishes" is well known to all who have made studies on the internal trematodes of fish. In this report he created 18 new genera and 14 new species; unfortunately in most cases the descriptions were meagre and accompanied by no supporting illustrations. Later workers have experienced great difficulty in trying to identify his forms and considerable confusion has resulted from attempts to assign the proper nomenclature for the species he described as new, as well as for the generic names he founded. It has been suggested that certain of his genera are not valid and that some of his identifications are not accurate. It is appreciated, of course, that owing to the unsettled state of trematode systematics at the time of his study and the difficulty he must have experienced in obtaining necessary literature, certain of his errors in identification could not be avoided. However, it must be admitted that the criticism of his contemporaries, in stating that he founded genera and species with far too inadequate a description, is valid.

The opportunity to study Dr. Stafford's slide collection has recently presented itself; although a few of the species he described are not present in the collection, the majority are present. Most are in a fair state of preservation, and some have retained their stain very well. A difficulty was encountered in attempting to connect the trematodes with his descriptions; in some cases the name of the parasite is not given, the slides being labelled only with

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Contribution from the Institute of Parasitology, McGill University, Macdonald College, Quebec, with financial assistance from the National Research Council of Canada.

[2] *Research Associate.*

the name of the host. In those cases where the names are given the genus is often represented by the letter, D. referring no doubt to *Distoma*. However, by comparing the morphology of the parasites with his description and taking into consideration the hosts and location of the parasites in the hosts, practically all his specimens could be assigned to their proper positions.

In the present report the species will be presented in systematic order with no attempt to follow the order in which they were presented by Stafford. In those cases where Stafford's nomenclature is wrong, his names are presented in parentheses underneath the proper name.

Description of Parasites

Family Buchephalidae

Prosorhynchus squamatus Ohdner, 1905

(Fig. 1)

(*Gasterostomum armatum*, of Stafford)

Host: *Brosmius brosme* (cusk), from the intestine?

Ohdner (13) pointed out that *Gasterostomum armatum* Molin, 1861, and *Gasterostomum armatum* of Olsson, 1868, and of Levinsen, 1881, represent different species. As *G. armatum* Molin, 1861, is synonymous with *Monostomum crucibulum* Rudolphi, 1819, Ohdner created the genus and species *Prosorhynchus squamatus* for the species represented by *G. armatum* of Levinsen and Olsson. He distinguished *Prosorhynchus* from *Bucephalus (Gasterostomum)* by its rostellum-like anterior sucker, the arc-like position of the vitellaria, and the character of the male genitalia.

The collection contains a single specimen from the cusk. It measures 2 mm. long by 0.8 mm. wide. The body is egg-shaped and covered with fine, closely set spines. The anterior sucker is rostellum-like in shape and measures 0.2 mm. by 0.18 mm. The mouth opening is just posterior to the middle of the body, and the ventral sucker surrounding it measures 0.16 mm. in diameter. The testes are slightly broader than long, situated in the third quarter of the body in tandem arrangement. The ovary is subspherical, situated just above the anterior testis and partially overlapping it. It is somewhat larger than the testes. The uterus is in the posterior half of the body and the genital pore near the posterior tip. The vitellaria form an arc midway between the rostellum and the mouth opening. The cirrus sac lies on the left side of the body; it is very large, measuring 0.64 by 0.3 mm. The eggs measure 0.032 by 0.041 mm. by 0.021 to 0.025 mm.

The specimen in the collection differs from the material described by Ohdner in the somewhat larger egg size, the larger cirrus sac, and the fact that the ovary is larger than the testes. While these characters may constitute the basis for the establishment of a new species the fact that only a single, considerably flattened specimen is available for study does not exclude the possibility that the differences may merely be variations within a species.

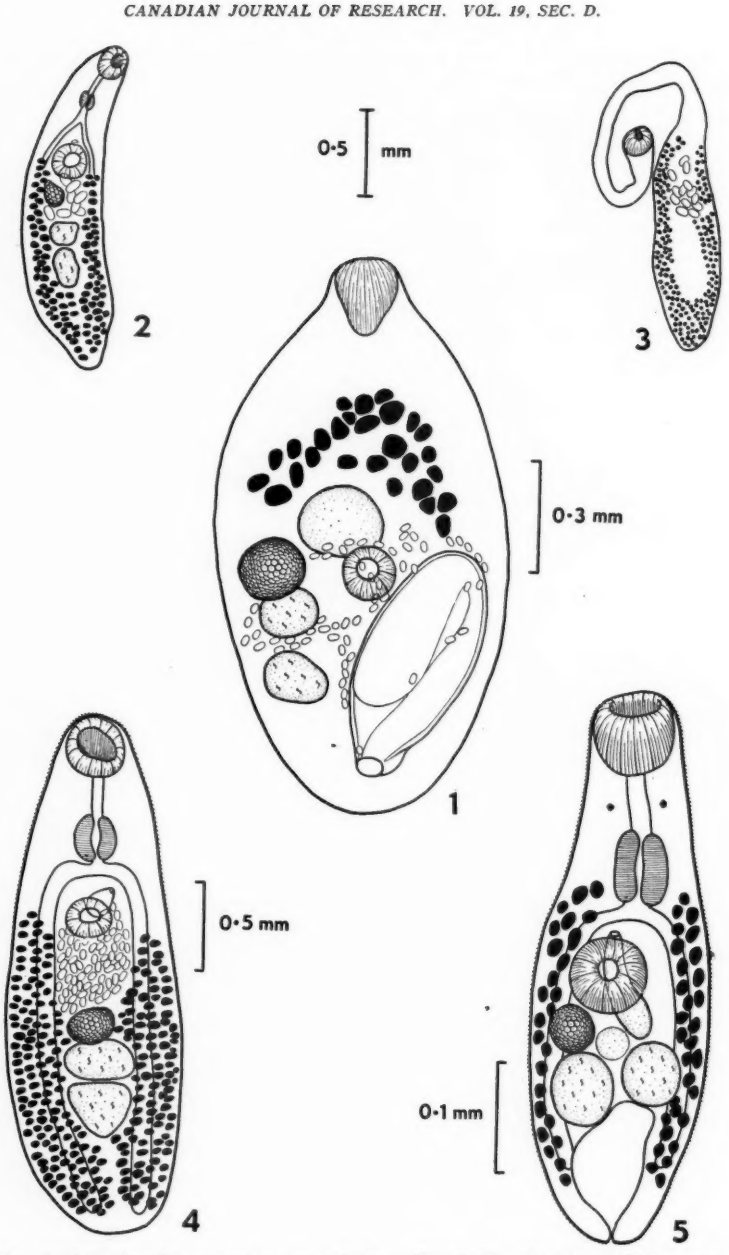

FIG. 1. *Prosorhynchus squamatus, ventral view.* FIG. 2. *Homalometron pallidum, ventral view.* FIG. 3. *Stenocollum fragile.* FIG. 4. *Lepidapedon rachion, ventral view.* FIG. 5. *Neophasis pusilla, ventral view.*

FAMILY ALLOCREADIIDAE

Crepidostomum cooperi Hopkins, 1932

(Fig. 6)

(*Crepidostomum laureatum*, of Stafford)

HOST: *Salvelinus fontinalis* (speckled trout), from the intestine.

The collection contains several well preserved specimens of this species ranging from 1.1 to 1.6 mm. in length. Hopkins (1) has pointed out that *Crepidostomum laureatum* Zeder, 1800, is synonymous with *C. farionis* Müller, 1784, which is quite distinct from the specimen in Stafford's collection.

Genus *Lepidapedon* Stafford, 1904

Lepidapedon rachion (Cobbold, 1858)

(Fig. 4)

HOST: *Melanogrammus aeglifinis* (haddock), from the intestine.

Stafford created the new genus *Lepidapedon* for *Distomum rachion* of Cobbold, but gave no description of it. Ohdner (13) gave a comprehensive description of this species and proposed the new genus *Lepodora*. However, Stafford's name holds priority and must be considered as correct.

The collection contains four specimens ranging from 1.5 to 3.1 mm. long by 0.4 to 1.0 mm. wide. None measure 5.0 mm. in length, the measurement given by Stafford for this species. The specimens are broadest in the third quarter of the body length, and narrow somewhat to the posterior end and more markedly to the anterior tip. The cuticula is covered with numerous spines which are more conspicuous at the anterior end of the body. The oral sucker is larger than the acetabulum and measures from 0.2 to 0.38 mm. in diameter. There is a long pre-pharynx and a conspicuous pharynx about the same size, being from 0.14 to 0.26 mm. in length. The oesophagus is very short and divides almost immediately into the intestinal caeca which extend to the posterior tip of the body. The acetabulum is situated in the second quarter of the body and measures from 0.13 to 0.35 mm. in diameter. The testes and ovary are roughly ovate in outline with the long axis horizontal. They are in tandem arrangement in the third quarter of the body with the ovary immediately anterior to the anterior testis. The uterus lies between the ovary and genital pore, which is situated midway between the intestinal bifurcation and the acetabulum, to the left of the mid-line. The broad cirrus sac overlaps the acetabulum for about half its diameter. The vitellarian follicles extend laterally from the acetabulum to the posterior tip of the body and fill in the post-testicular body space. The eggs measure 0.070 to 0.078 mm. by 0.042 mm.

Homalometron pallidum Stafford, 1904

(Fig. 2)

HOST: *Fundulus heteroclitus* (killifish), from the intestine.

The generic and specific names of this parasite were proposed by Stafford who gave no description but referred to the description of Linton (4, 5).

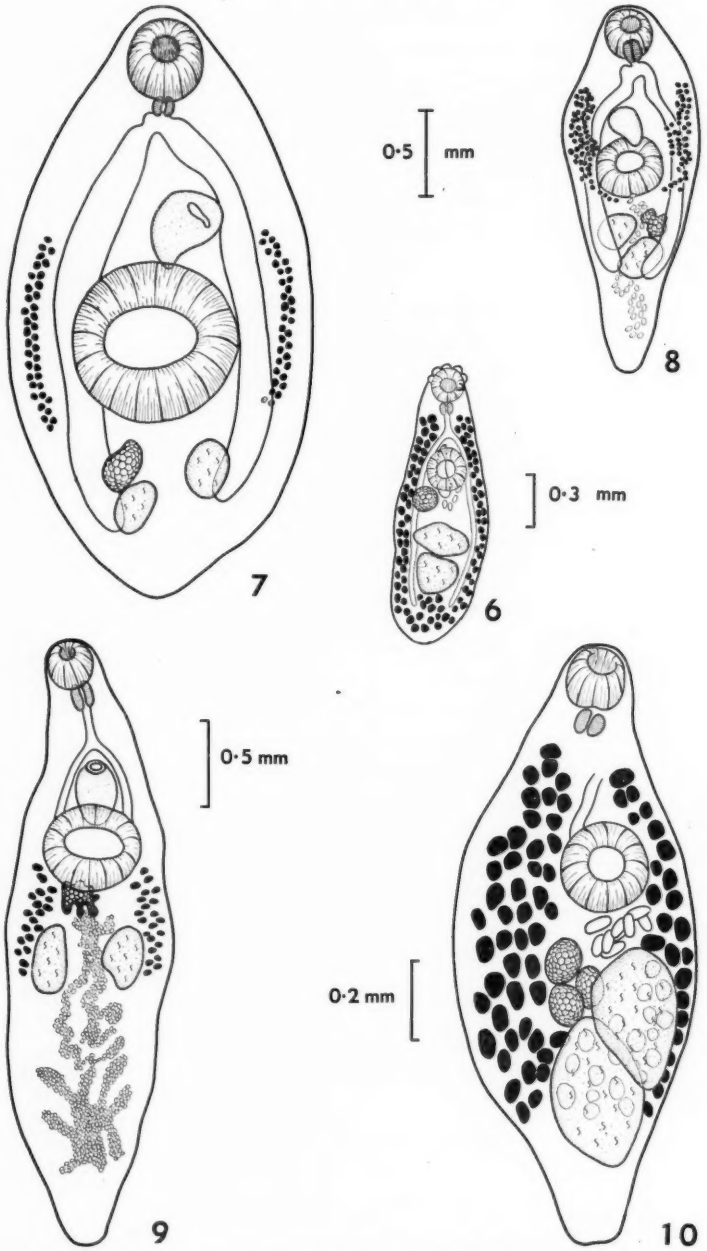

FIG. 6. *Crepidostomum cooperi, ventral view.* FIG. 7. *Fellodistomum fellis, ventral view.* FIG. 8. *Fellodistomum agnotum, dorsal view.* FIG. 9. *Steringophora furciger, ventral view.* FIG. 10. *Stenakron vetustum, ventral view.*

Looss (7) considers *Homalometron* synonymous with *Lepocreadium* (Stossich, 1903). Manter (10) considers the two genera closely related but differentiable on the basis of the position of the genital pore, which he states is median in *Homalometron* and to the left of the mid-line in *Lepocreadium*, and the presence of a cirrus pouch in the latter genus and its absence in the former. However, Linton (4) states that the genital pore is median in *Lepocreadium trulla*, and his figures of this species show the pore to be in a median position. He states that certain specimens, which he assigns to the species *L. levinseni*, but which he suspects may belong to another species, do not have a cirrus pouch. It thus seems not unlikely that *Homalometron* may be a synonym of *Lepocreadium* Stossich, 1904. However, until all species are carefully compared it seems wiser to consider them as distinct genera for the time being.

The specimens in the collection are small, narrow forms measuring 1.76 to 1.94 mm. by 0.040 to 0.048 mm. They are broadest in the region posterior to the middle and taper somewhat posteriorly and more markedly to the anterior tip. The cuticula shows evidence of being spinose although most of the spines have been lost. The oral sucker is terminal and subspherical in outline; it leads by an obvious pre-pharynx to a conspicuous pharynx of about the same length. The short oesophagus divides into the intestinal caeca which extend to near the posterior tip of the body. The acetabulum is situated about one-third of the body length from the anterior tip. It is somewhat larger than the oral sucker. The testes are in tandem arrangement in the second half of the body. They are subspherical to oval in shape and may be somewhat irregular in outline. The ovary is anterior to the anterior testis and is displaced slightly to the right. The uterus leads back to the anterior margin of the anterior testis rather than forward to the genital pore situated immediately anterior to the acetabulum on the mid-line. There is no cirrus pouch. The vitellaria extend laterally from the posterior half of the acetabulum to the posterior tip of the body, and fill in the post-testicular space. An average specimen shows the following measurements: length 1.9 mm., width 0.5 mm., oral sucker 0.18 by 0.15 mm., acetabulum 0.23 mm. in diameter, pharynx 0.095 mm. long, eggs 0.092 to 0.11 mm. by 0.54 mm.

Neophasis pusilla Stafford, 1904

(Fig. 5)

HOST: *Annarhichas lupus* (wolf fish), from the urinary bladder.

Stafford created the new genus and species *Neophasis pusilla* for a small trematode he obtained from the urinary bladder of the wolf fish. Lebour (3) makes brief mention and gives diagrams of a trematode from the stomach of the wolf fish. In a later report (1909) she refers it to the genus *Acanthopsolus* of Ohdner (13) under the name *A. lageniformis*. Ohdner (14) considers *A. lageniformis* a synonym of *Neophasis pusilla* but he suppresses the genus *Neophasis* in favour of *Acanthopsolus* and retains Lebour's name of *A. lageniformis* for this parasite. Although the author has not seen Lebour's later report (1909), her diagrams of this parasite in the earlier publication (3)

suggest it to be a different species from *N. pusilla*. In Lebour's species the genital pore is situated on a level with the pharynx on the left side of the body, and the cirrus pouch lies wholly in front of the acetabulum, whereas Stafford's specimens have the genital pore immediately anterior to the acetabulum on the mid-line, and the cirrus pouch extends some distance below the posterior margin of the acetabulum. The author does not believe Ohdner (14) is justified in suppressing the genus name *Neophasis*, as the characters presented by Stafford are sufficient for the recognition of the genus. *Acanthopsolus* is therefore considered a synonym of *Neophasis* which contains the following three species: *N. pusilla* (Stafford, 1904) as the type species, *N. lageniformis* (Lebour, 1909), and *N. oculatus* Ohdner, 1905.

Poche (17), Fuhrmann (2), and more recently Ward (22) all place the genus *Neophasis* (*Acanthopsolus*) in the family Acanthocolpidae, although Ward (22, pp. 509-521) states that it disagrees with the family concept, and erects the subfamily Acanthopsolinae for its reception. However, in general morphology this genus shows a much closer relationship to the Allocreadiidae than it does to the Acanthocolpidae. *Neophasis* is accordingly considered by the writer as belonging to the family Allocreadiidae and tentatively placed in the subfamily Lepocreadiinae.

The material in Stafford's collection consists of six specimens, the largest of which measures 0.60 mm. in length. As Stafford has stated they are shaped like an Indian club with a rounded posterior end and a square cut anterior tip, which ends in the large oral sucker. The entire cuticula is covered with prominent closely set spines. The terminal oral sucker is subspherical. It leads by an elongate pre-pharynx to a large pharynx that measures somewhat less than the oral sucker. There is no apparent oesophagus, and the pharynx leads directly into the intestinal caeca which extend to within a short distance of the posterior tip of the body. The acetabulum is spherical, slightly smaller than the oral sucker, and is situated about halfway down the body length. There is a prominent eyespot on either side of the pre-pharynx. The testes are oval to subspherical in outline, side by side in a slightly oblique arrangement; the left testis is the more anterior. The ovary is subspherical, and is situated between the right testis and the acetabulum. The uterus extends from the anterior margin of the testes to the genital pore, which is situated on the mid-line immediately anterior to the acetabulum. The cirrus pouch extends posterior to the acetabulum, almost to the level of the testes. The vitellaria extend laterally from the pharynx to near the posterior tip. The excretory bladder is small and sac-shaped. The exact size of the eggs could not be determined as they were badly collapsed, but they appear to be quite large and to measure roughly about 0.08 mm. in length. Measurements of an average specimen are as follows: length 0.52 mm., width 0.18 mm., oral sucker 0.076 mm. in diameter, ventral sucker 0.071 mm. in diameter, pharynx 0.067 mm. long by 0.054 mm. wide, right testis 0.070 mm. in diameter, left testis 0.063 mm. in diameter, ovary 0.044 mm. in diameter.

Genus *Podocotyle* Stiles and Hassall, 1898

Synonym: *Sinistroporus* Stafford, 1904

Podocotyle atoman Rudolphi, 1802

(*Sinistroporus simplex* in part, of Stafford)

(Fig. 14)

HOSTS: *Cottus scorpius* (sculpin)
Scomber scombrus (mackerel)
Sebastes marinus (rose fish), from the intestine.

Stafford created the genus *Sinistroporus* for *Distomum simplex* Rudolphi. Ohdner (13) has pointed out that *D. simplex* is a synonym of both *D. angulatum* and *D. atoman*. He further states that *D. angulatum* which was made the type species of the genus *Podocotyle* by Stiles and Hassall (20) cannot be retained because it was inadequately described, and because the type material has been lost. Thus *P. atoman* becomes the proper name of this species and the type of the genus.

The collection contains 12 specimens of this species, some in a good state of preservation. They agree quite well with the detailed description of Ohdner (13). Specimens range from 1.45 to 3.81 mm. in length.

Podocotyle reflexa Creplin, 1825

(Fig. 11)

(*Sinistroporus productus* and *S. simplex* in part, of Stafford)

HOSTS: *Hemitripterus americanus* (sea raven)
Urophycis tenuis (hake), from the intestine.

Stafford included two species in his genus *Sinistroporus: S. simplex* and *S. productus*. He described *S. productus* which he named as a new species, as follows: "6.93 × 0.60, long and narrow. Testes elliptical and some distance apart in the longitudinal axis of the worm." This is his entire description, and while it is certainly not sufficiently detailed to differentiate a new species there is little doubt that Stafford actually was dealing with a species different from his *P. simplex*. None of the slides in the collection are labelled *S. productus*. However, he gives the host of *S. productus* as *Hemitripterus americanus*, and all the specimens of *Podocotyle* that he has in the collection from *H. americanus* prove upon examination to be *P. reflexa*.

Ohdner (13) separates *P. reflexa* and *P. olssoni* by the proportion of the body width to length, the proportion of the length of the oesophagus to that of the pharnyx, the extent of the cirrus sac, and the outline of the cross-section of the body. According to these characters the specimens from the sea raven agree with the species description of *P. reflexa* as the proportion of the body width to length ranges from 1 : 9 to 1 : 12, and the cirrus sac does not reach to nearly half the distance between the acetabulum and the ovary. The specimen from the hake shows some resemblances to *P. olssoni* in that the proportion of the body width to length is just slightly over 1 : 7, and the

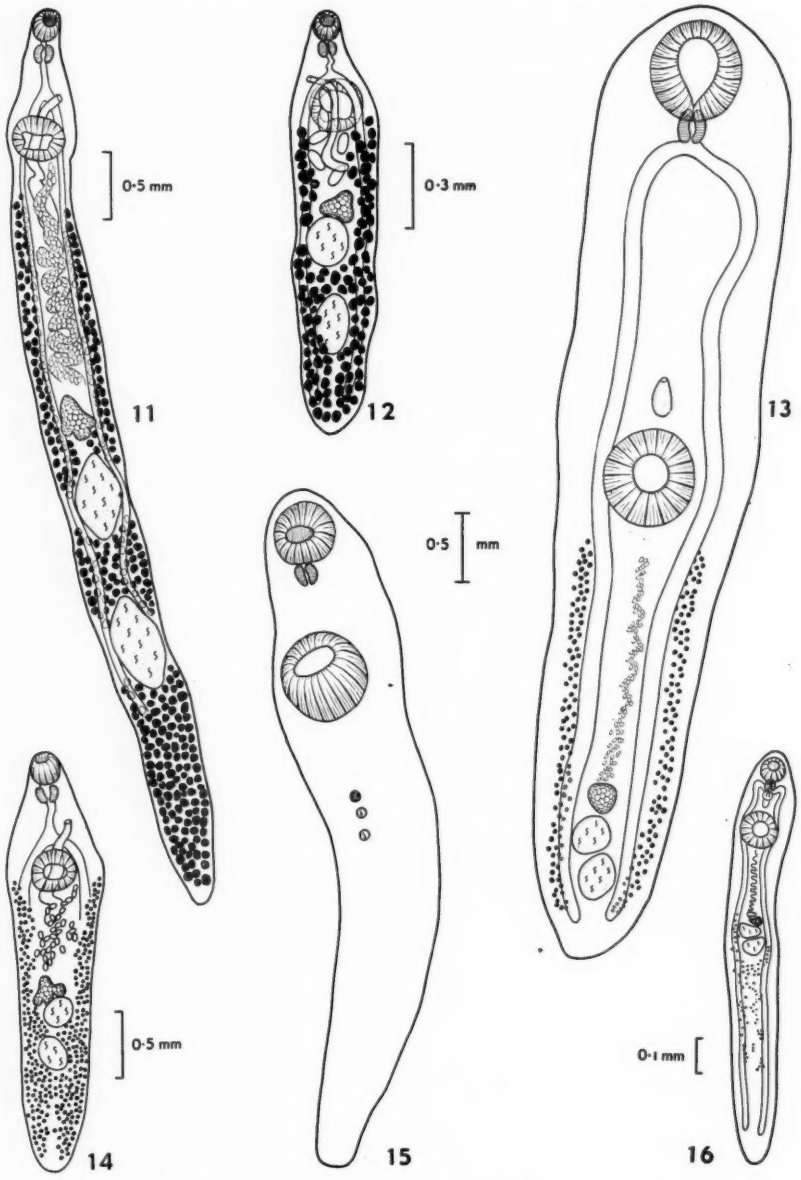

FIG. 11. *Podocotyle reflexa, ventral view.* FIG. 12. *Podocotyle staffordi, dorsal view.* FIG. 13. *Azygia angusticauda, ventral view.* FIG. 14. *Podocotyle atoman, ventral view.* FIG. 15. *Otodistomum cestoides (Xenodistomum melanocystis of Stafford), immature.* FIG. 16. *Otodistomum cestoides, ventral view.*

more anterior position of the ovary suggests that the cirrus sac, which cannot be seen, may reach half-way the distance from the acetabulum to the ovary. On the other hand the oesophagus in this specimen, as in *P. reflexa*, is definitely longer than the pharynx. As the specimen from the hake showed considerable contraction at the anterior end, the similarities to *P. olssoni* were very likely due to this. The difficulty encountered in attempting to assign this specimen to the proper species points out the desirability of using characters other than those of size alone in differentiating trematode species.

Members of this species are long, narrow, parallel-sided forms. The specimen from the hake was the smallest, measuring 3.6 mm. long. Mature specimens from the sea raven measured from 4.5 to 7.5 mm. in length. The oral sucker is terminal and about half the size of the subspherical acetabulum which is situated not far from the anterior tip of the body. There is a short pre-pharynx, a well developed pharynx, and an oesophagus which is usually longer than the pharynx. The oesophagus divides a short distance anterior to the acetabulum, and the narrow intestinal caeca extend to near the posterior tip of the body. The ovary is situated approximately halfway down the body length, or may be slightly anterior or posterior to this position. It usually shows three lobes. The uterus lies anterior to the ovary and leads to the genital pore situated on a level with the intestinal bifurcation, on the left side of the body. The vitellaria extend laterally a short distance behind the acetabulum to the posterior tip of the body, and, as is characteristic of this species, fill in the inter-testicular spaces, and are discontinued laterally in the region of the testes. The testes are large, spindle to oval shaped, situated in the posterior half of the body. The elongate cirrus pouch extends about a third of the distance from the acetabulum to the ovary. The measurements of one of the larger specimens is as follows: length 7.0 mm., width 0.59 mm., oral sucker 0.2 mm. in diameter, pharynx 0.17 mm. long, oesophagus 0.23 mm. long, acetabulum 0.33 by 0.41 mm., ovary 0.37 by 0.27 mm., anterior testis 0.68 by 0.39 mm., posterior testis 0.75 by 0.41 mm., eggs 0.084 by 0.042 mm.

Podocotyle staffordi sp. nov.

(Fig. 12)

(*Sinistroporus simplex* in part, of Stafford)

HOST: *Gasterosteus aculeatus* (stickleback), from the intestine.

The collection contains a number of small specimens of a *Podocotyle* species from the stickleback. They are labelled *D. simplex*, but upon study showed characters that place them in a distinct species for which the name *P. staffordi* is proposed.

Podocotyle staffordi is most closely related to *P. olssoni*, which it resembles a great deal, but from which it differs by the character of its vitellaria and its smaller size. *P. staffordi* has the vitellaria filling the inter-testicular space but unlike *P. olssoni* the vitellaria are not discontinued laterally in the region

of the testes. Manter (10) refers to specimens of *P. olssoni* from the cod that appear to resemble *P. staffordi* in their size and in the fact that the lateral break in the vitellaria in the region of the testes does not always occur. The relationship of these forms to *P. staffordi* cannot be determined until the two forms are carefully compared. Nicoll (11) reported specimens of *P. atoman*, from the stickleback, which are very similar in size range to *P. staffordi*. However, he does not give the distribution of the vitellaria, and it seems likely that he was dealing with specimens of *P. staffordi*.

Specimens in the collection measure from 1.07 to 1.51 mm. in length, and are from five to six times as long as broad. The oral sucker is terminal, and spherical to subspherical in outline. The acetabulum is situated from one-fifth to one-quarter the distance from the anterior tip; it is spherical and about twice the diameter of the oral sucker. There is a short pre-pharynx, a well developed pharynx, and an oesophagus that is somewhat longer than the pharynx. The narrow intestinal caeca extend to near the posterior tip of the body. The three-lobed ovary is situated just anterior to the middle of the body. The uterus passes anterior from the ovary to the genital pore situated on a plane with the intestinal bifurcation, on the left side of the body. The testes are situated in the second half of the body in tandem arrangement; they are oval in outline and separated by several rows of vitellarian follicles. The vitellaria extend laterally from the posterior margin of the acetabulum to the posterior tip, filling the inter- and post-testicular spaces; they are not interrupted laterally in the region of the testes. The cirrus pouch extends about half the distance from the acetabulum to the ovary. The type specimen shows the following measurements: length 1.42 mm., width 0.28 mm., oral sucker 0.084 mm. in diameter, acetabulum 0.17 mm. in diameter, pharynx 0.052 mm. long, ovary 0.1 by 0.1 mm., anterior testis 0.16 by 0.13 mm., posterior testis 0.18 by 0.11 mm., eggs 0.091 by 0.045 mm.

Stenakron vetustum Stafford, 1904

(Fig. 10)

Host: *Hemitripterus americanus* (sea raven), from the intestine.

Stafford proposed the genus and species names *Stenakron vetustum* for a trematode from *Limanda ferruginea* which Linton (5) described but did not name. Stafford gives no description but refers to the description of Linton as follows:—

"Body smooth, fusiform, thickset about the middle tapering nearly equally to each end. Anterior sucker subterminal, circular, aperture somewhat triangular in preserved specimens, acetabulum, a little in front of the middle, larger than oral sucker, aperture nearly circular. Pharynx subglobular, close to oral sucker, oesophagus distinct. Intestinal rami simple, extending to ovary. Vitellaria distributed in the middle regions of the body from testes

to pharynx. Testes two, rather large, placed a little diagonally on the median line near posterior end of body. Ovary smaller than testes, subglobular or slightly lobed, situated in front of the anterior testis and to the right. Ova, few, large, in front of ovary. Cirrus pouch to the right of acetabulum. Genital aperture about half-way between acetabulum and oral sucker, to right of median line, at about midway between pharynx and acetabulum. Dimensions of living specimen, in millimetres; length 2.57; diameter, anterior 0.25, middle 0.93; posterior 0.19; diameter of oral sucker 0.21, of acetabulum 0.36; anterior testis, length 0.43, breadth 0.36; posterior testis, length 0.43, breadth 0.37; ova, 0.065 and 0.041 in the two principle diameters."

The collection contains two specimens, one of which is badly contracted. Both specimens are overstained and the details of the alimentary tract and the cirrus pouch cannot be made out. The organs that can be seen agree quite well with Linton's description. The one important exception is in the character of the ovary. In the one specimen in which the ovary can be seen clearly it consists of three separate and distinct oval parts. Thus there are apparently three ovaries. Whether this holds true for all specimens of this species cannot of course be stated until further specimens are studied. However, Linton's diagrams of this species could be interpreted as having two ovaries. Stafford's specimens differ further from the Linton's description in the larger size of the eggs and the testes. They show the following measurements: body sizes 1.60 by 0.70 mm. and 1.50 by 0.72 mm. respectively, diameters of oral suckers 0.16 and 0.19 mm., diameters of acetabula 0.24 and 0.30 mm., pharynx 0.09 mm., anterior testis 0.39 by 0.24 mm., posterior testis 0.41 by 0.26 mm., eggs 0.077 by 0.036 mm.

Stenakron vetustum has not been assigned to any definite group of trematodes and Poche (17) lists it under "unclassified general". In its general morphology it agrees mostly nearly with members of the family Allocreadiidae where it probably belongs.

Genus *Plagioporus* Stafford, 1904

Synonyms: *Lebouria* Nicoll, 1909

Caudotestis Yamaguti, 1934

Plagioporus serotinus Stafford, 1904

HOST: Moxostomata sp. (red horse sucker).

Stafford gave the name *Plagioporus serotinus* to a trematode he obtained from the intestine of the red horse sucker. As the author has pointed out (9) this species is valid as is the genus, and *Lebouria* Nicoll, 1909, and *Caudotestis* Yamaguti, 1934, are synonyms of *Plagioporus*.

The material in the collection consists of three specimens none of which bear eggs. They range from 1.34 to 1.65 mm. in length and are approximately one-fourth as broad as long. The species has been fully described elsewhere (9) and will not be discussed further here.

Stenocollum fragile (Linton, 1900)

(Fig. 3)

HOST: *Mola mola* (sunfish), from the intestine.

The genus *Stenocollum* was created by Stafford to include *Distomum fragile* (Linton, 1900). Stafford gave no description but referred to the description of Linton which reads as follows:—

"Body unarmed, fusiform from acetabulum back, depressed, neck elongated, slender, cylindrical, slightly enlarged at mouth. Acetabulum a little larger than mouth, subglobular, at base of neck sessile; mouth terminal, or nearly so; pharnyx subglobular, situated a distance equal to twice its length or more behind the posterior edge of the oral sucker, followed by a slender oesophagus; intestinal crura simple, beginning in the neck about half-way between the pharynx and acetabulum, extending to near the posterior end of the body; testes two, median, approximate, situated near the posterior end of the body, a little longer than broad; ovary subtriangular in outline, lying immediately in front of the anterior testis and a little to the right; cirrus and cirrus pouch immediately in front of the acetabulum and to the left; vitellaria very abundant, appearing in subangular masses at posterior end and along dorsal and lateral regions of the body to and even in front of the acetabulum; uterine folds between acetabulum and ovary; ova relatively large and in moderate number."

"Dimensions of mounted specimen in millimetres: length 1.78, diameter of anterior sucker 0.10, diameter of neck behind mouth 0.07, diameter of acetabulum 0.24, greatest diameter 0.33, distance of acetabulum from anterior end 0.71, diameter of acetabulum 0.14, length of testis 0.17, breadth 0.14, diameter of ovary 0.10, longer diameter of ovum 0.069, shorter diameter 0.038, length of pharynx 0.06, distance between pharynx and anterior sucker 0.15."

The collection contains several specimens of this species, which are, unfortunately, badly faded. Only the body shape, the distribution of the vitellaria and the uterus, and the size of the eggs, can be made out with certainty. For this reason very little can be added to Linton's description. However, the specimens in the collection measure, on the average, slightly more than 3 mm. in length with the proportion of the thin neck region to the thicker main part of the body being as 1.5–2 : 1. The width of the body at the widest part ranges from 0.33 to 0.41 mm. The eggs measure 0.0612 to 0.0693 mm. in length by 0.0386 to 0.0408 mm. in width.

Poche (17) included the genus *Stenocollum* with his genera *Fasciolidorum sedis incertae*. From the characters presented by Linton (4) it can readily be included in the family Allocreadiidae. It shows many resemblances to the genus *Anallocreadium* Simer, 1929, and upon further study it may be possible to demonstrate that *S. fragile* does not have a true cirrus pouch, as is the case in *Anallocreadium*.

FAMILY HEMIURIIDAE

Hemiurus levinseni Ohdner, 1905

(Fig. 19)

(*Hemiurus appendiculatus* in part, of Stafford)

HOSTS: *Gadus callarias* (cod)
Clupea harengus (herring), from the oesophagus and stomach.

The collection contains 10 specimens of this species labelled *D. ocreatum*, but which, from the host list and the fact that he makes no mention of *D. ocreatum*, Stafford must have included in his species *H. appendiculatus*. The specimens from the cod are larger than those from the herring, an average specimen measuring 2.36 mm. in length. An average specimen from the herring measures 1.45 mm. in length. The specimens can be recognized as *H. levinseni* by the fact that the two suckers are approximately equal, while they are obviously unequal in the species *H. appendiculatus* and *H. communis*.

Brachyphallus crenatus Rudolphi, 1802

(Fig. 18)

(*Hemiurus appendiculatus* in part, of Stafford)

HOSTS: *Salmo salar* (salmon)
Hippoglossus hippoglossus (halibut)
Osmerus mordax (smelt)
Reinhardtius hippoglossoides (greenland turbot), from oesophagus, stomach, and intestine.

The 12 specimens in the collection were all labelled *D. appendiculatus* and were apparently placed by Stafford together with *H. levinseni* in the species *H. appendiculatus*. The average specimens measure from 1.5 to 2.0 mm. in length in an extended condition. However, the single specimen from the Greenland turbot measures 3.2 mm. in length. The width is about one-fifth the length. While the specimens vary in the structure of the vitellaria, some being lobed and others smooth-margined, there appears to be only one species and not two as stated by Looss (7).

Derogenes varicus Muller, 1788

(Fig. 17)

HOSTS: *Salmo salar* (salmon)
Gadus callarius (cod)
Osmerus mordax (smelt)
Anguilla chrysypa (eel)
Cottus scorpius (sculpin)
Hippoglossus hippoglossus (halibut)
Reinhardtius hippoglossoides (Greenland turbot)
Melanogrammus aegelfinus (haddock), from the oesophagus and stomach.

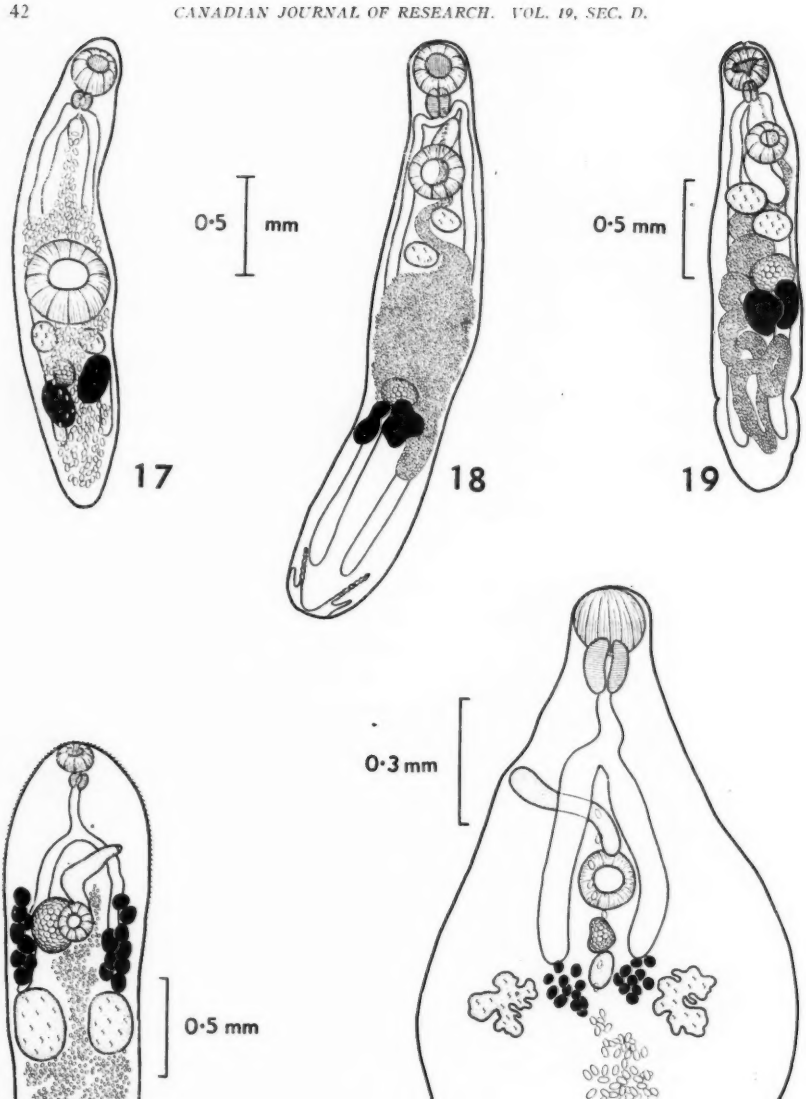

FIG. 17. *Derogenes varicus, ventral view.* FIG. 18. *Brachyphallus crenatus, ventral view.*
FIG. 19. *Hemiurus levinseni, ventral view.* FIG. 20. *Steganoderma formosum, ventral view.*
FIG. 21. *Lepidophylum steenstrupi, dorsal view.*

This species is the most numerous in the collection. Specimens range from 1.3 to 3.0 mm. in length, and in morphology agree essentially with the excellent description of Ohdner (13).

Specimens of the species *Derogenes plenus*, created by Stafford, could not be found in the collection.

Family Fellodistomidae

Genus *Fellodistomum* Stafford, 1904

Fellodistomum fellis Olsson, 1868

(*Fellodistomum incisum*, of Stafford)

(Fig. 7)

Host: *Annahrichas lupus* (wolf fish).

Although Nicoll (11) accepts Stafford's name for the genus *Fellodistomum*, he points out that *F. fellis* is a different species from *Distomum incisum* Rudolphi, 1809. Moreover because of Stafford's description stating that the ovary is on the left side of the body, and the testes large and spherical in outline, Nicoll doubts whether he was dealing with *F. fellis*. The specimen in Stafford's collection, however, shows the ovary on the right side of the body, and the testes obliquely oval; thus there is little doubt that it was *F. fellis*.

The collection contains a single immature specimen, which measures 3.48 by 1.8 mm. It is broadly ovate in outline. The oral sucker is subterminal, and measures 0.45 mm. in diameter. The acetabulum is situated slightly posterior to the middle of the body; its diameter is more than twice that of the oral sucker, it is spherical in outline, and measures 0.98 mm. in diameter. The pharynx is 0.16 mm. long. There is no pre-pharynx or oesophagus. The broad intestinal caeca extend to near the posterior margins of the testes. The testes are situated midway between the acetabulum and the posterior extremity. They are side by side with the left testis in a slightly more posterior position. They are obliquely oval in outline and are separated by their own width. The ovary is situated immediately anterior to the right testis on the right side of the body. The genital opening lies slightly to the left of the mid-line, midway between the anterior margin of the acetabulum and the intestinal bifurcation. The broad cirrus pouch lies almost wholly in front of the acetabulum. The vitellaria form a narrow band on either side of the acetabulum, extending slightly anterior and posterior to this organ.

Fellodistomum agnotum Nicoll, 1909

(*Fellodistomum incisum* of Stafford?)

(Fig. 8)

Host: *Annahrichas lupus* (wolf fish), from the gall bladder.

The collection contains two mature and one immature specimen. Whether Stafford includes these in his *F. incisum* cannot be stated definitely as the

slides are not labelled. However, as he makes no special mention of them it seems most likely that he included them in *F. incisum*.

Fellodistomum agnotum was described as a new species by Nicoll (11), who differentiated it from *F. fellis* by the smaller size of the ventral as compared to the oral sucker, the more dorsal position of the vitellaria, and the more elongate body. The specimens in the collection are roughly spindle-shaped forms somewhat attenuated anteriorly. The oral sucker is spherical and subterminal. The acetabulum is larger than the oral sucker but its diameter is less than twice that of the latter, it is usually flattened in the antero-posterior axis. There is a well developed pharynx which leads directly into the broad intestinal caeca. The intestinal caeca extend to the posterior margin of the right testis. The two somewhat lobed testes are situated in the third quarter of the body in oblique arrangement, the left testis being more anteriorly placed. The lobed ovary is situated immediately anterior to the right testis. The uterus passes back to the posterior tip of the body, then passes forward to the genital pore situated on the right of the mid-line, midway between the acetabulum and the intestinal bifurcation. The cirrus pouch overlaps the acetabulum for a short distance. The vitellaria extend laterally from the intestinal bifurcation to the posterior margin of the acetabulum. Measurements of the largest mature specimen are as follows: length 2.9 mm., width 1.09 mm., oral sucker 0.29 mm. in diameter, pharynx 0.14 mm. long, acetabulum 0.42 mm. by 0.46 mm., eggs 0.044 by 0.024 mm.

The material in the collection differs from Nicoll's description in the more posterior extent of the vitellaria and the oblique arrangement of the testes, characters that are probably only variations within a species.

Steringophora furciger Olsson, 1868

(Fig. 9)

(*Leioderma furcigerum* of Stafford)

HOSTS: *Reinhardtius hippoglossoides* (Greenland turbot)
Pseudopleuronectes americanus (winter flounder)
Cryptacanthodes maculatus (wry mouth).

Stafford (19) created the genus *Leioderma* for *Distoma furciger* Olsson. However, *Leioderma* is preoccupied by *Leiodermia* Willemoes-Suhm, 1873, thus *Steringophora* Ohdner, 1905, becomes the proper genus.

The collection contains 10 specimens. Mature specimens vary from 1.24 to 4.0 mm. in length and are from one-fifth to one-third as wide as long. The oral sucker is subspherical, measuring 0.35 to 0.41 mm. in diameter, and is situated one-third of the body length from the anterior tip of the ventral sucker; it is broader than long and measures 0.4 by 0.6 mm. The pharynx is 0.18 mm. long. The oesophagus is slightly longer than the pharynx. The intestinal caeca extend a short distance posterior to the testes. The testes are oval in outline and are arranged side by side at approximately the middle of the body. The ovary is a highly lobed structure, situated slightly

to the right of the mid-line between the testes and the acetabulum, and usually overlapping the latter organ to a slight extent. The uterus extends back to the posterior tip of the body. The genital pore lies on the mid-line, a short distance anterior to the acetabulum. The cirrus pouch is broadly oval in outline. It overlaps the acetabulum to about one-fifth of its length. The vitellaria are limited to a space between the posterior third of the acetabulum and the posterior margins of the testes. The eggs measure 0.055 by 0.029 mm.

Family Zoogonidae

Steganoderma formosum Stafford, 1904

(Fig. 20)

Host: *Hippoglossus hippoglossus* (halibut), from the intestine.

This species was described by Stafford who created for it the new genus *Steganoderma*. Manter (10) points out the close resemblance of *Steganoderma* to *Lecithostaphylus* Ohdner, 1911, but differentiates them on the basis of the difference in shape of the cirrus pouch, the difference in size of the pharynx, the oesophagus, and the excretory bladder, and the presence or absence of a Laurer's canal pore. It is doubtful whether these characters are sufficiently constant or important to justify the retention of the genus *Lecithostaphylus*.

The collection contains a single specimen, which measures 2.69 mm. by 0.76 mm. It is rounded at the anterior tip and somewhat truncate at the posterior extremity. The cuticula is covered with small closely set spines that are more prominent in the anterior part of the body. The oral sucker is flattened antero-posteriorly and measures 0.2 by 0.15 mm. The pharynx is small, measuring 0.063 mm. in length. The length of the oesophagus is more than twice that of the pharynx, and it divides into the intestinal caeca about half-way between the suckers. The intestinal caeca extend to the anterior margins of the testes. The acetabulum is situated about two-fifths of the body length from the anterior tip; it is subspherical and measures 0.2 mm. in diameter. The testes are arranged side by side just below the middle of the body length, and are broadly ovate in shape. The right testis is somewhat the larger, measuring 0.37 by 0.29 mm., whereas the left testis measures 0.35 by 0.25 mm. The ovary is ovate in shape; it is situated to the right of the mid-line, almost on a level with the acetabulum and partially overlapping that organ. The uterus almost fills the entire post-testicular region. The genital pore is situated on the left side of the body on a level with the intestinal bifurcation. The vitellarian follicles are large and few in number; they extend laterally from slightly anterior of the acetabulum to the anterior margin of the testes. The narrow cirrus pouch overlaps the acetabulum a very short distance. The eggs measure 0.036 by 0.017 mm.

Family Azygiidae

Genus *Azygia* Looss, 1899

Synonyms: *Megadistomum* Stafford, 1904
Mimodistomum Stafford, 1904

Stafford created two new genera and one new species for the members of the genus *Azygia* he found in freshwater fish. Ohdner (16) correctly points out that there are insufficient grounds for the creation of the two new genera, and considers *Megadistomum* and *Mimodistomum* synonymous with *Azygia*.

Azygia longa (*Leidy, 1851*)

(*Mimodistomum longum* and *Azygia tereticolle*, of Stafford)

Hosts: *Esox masquinongy* (muskallunge)
Lota maculosa (ling), from the stomach.

The collection contains two specimens of this species, one from the muskallunge and one from the ling. The specimen from the muskallunge is undoubtedly Stafford's *Megadistomum longum*. It is a large well preserved specimen measuring 18.5 by 1.2 mm. It does not contain any eggs and is undoubtedly referred to by Stafford when he states with regard to this species: "Alcoholic specimens measuring 18 mm. in length and over 1.0 mm. wide, have genital glands developed but no eggs."

The specimen from the ling, though not labelled, is undoubtedly *A. tereticolle* of Stafford. The specimen measures 6.5 by 0.5 mm. and contains eggs. Manter (10) points out that *A. tereticolle* is synonymous with *A. longa*.

Azygia angusticauda (Stafford, 1904)

(Fig. 13)

(*Mimodistomum angusticaudum*, of Stafford)

Host: *Lota maculosa* (ling), from the stomach.

The collection contains two mature and several immature specimens. The two mature specimens measure 7.25 by 1.65 mm. and 7.0 by 1.2 mm., respectively. The oral sucker is terminal, spherical to subspherical in outline. There is a well developed pharynx that opens directly into the intestinal caeca. The intestinal caeca extend to the posterior tip of the body. The acetabulum is about the same size as the oral sucker, or may be slightly smaller, and is situated about half-way down the body length. The genitalia are crowded towards the posterior tip of the body, the testes being posterior to the ovary and in tandem to oblique arrangement. The ovary is immediately anterior to the anterior testis. The uterus lies between the ovary and the genital pore, which is situated on the mid-line a short distance anterior to the acetabulum. The vitellaria extend laterally from a short distance posterior to the acetabulum to the posterior end of the body. One of the specimens shows the following measurements: length 7.25 mm., width 1.65 mm., oral sucker 0.83 by 0.72 mm., acetabulum 0.72 mm. diameter, pharynx 0.29 mm. long, anterior testis 0.35 mm. diameter, posterior testis 0.39 by 0.33 mm.

Genus *Otodistomum* Stafford, 1904

Otodistomum cestoides van Beneden, 1871

(Fig. 16)

(*Otodistomum veliporum* and *Xenodistomum melanocystis*, of Stafford)

HOST: *Raia stabuliforis* (barn door skate), from the stomach and intestine.

Stafford founded the genus *Otodistomum* to include what he thought was *D. veliporum* Creplin, 1837. Ohdner (16) and Manter (10) believe Stafford's species to be *O. cestoides*. The more constant differences between these two species appears to be the egg size and the size of the genital papilla. Unfortunately Stafford's specimens are all immature. However, in one specimen a prominent genital papilla, characteristic of *O. cestoides*, can be seen. Furthermore, from their distribution and host it seems most likely that Stafford's specimens are *O. cestoides*.

The collection contains three immature specimens, the largest of which measures 12.5 by 1.6 mm. The measurements given below refer to this specimen. The body is parallel-sided with rounded ends. The oral sucker is subterminal, flattened in the antero-posterior axis, and measures 0.72 by 0.79 mm. The pharynx is well developed, measuring 0.31 mm. in length. The caeca originate immediately behind the pharynx and extend to the posterior end of the body. The acetabulum is situated about one-fifth the length of the body from the anterior tip; it is larger than the oral sucker, measuring 1.14 by 1.24 mm. The genitalia are situated slightly anterior to the middle of the body, the testes are in approximate tandem arrangement, and the ovary lies immediately anterior to the anterior testis and slightly to the left of the mid-line. The uterus lies between the ovary and the genital pore, which is situated just posterior to the pharynx. The vitellaria consist of small scattered follicles concentrated for the most part posterior to the genitalia. The genital opening has a conspicuous protruding papilla.

Stafford created the genus and species *Xenodistomum melanocystis* for immature trematodes he found encysted in the stomach wall of *Lophius piscatorius* (goose fish). From the general appearance of the immature specimens in the collection from the goose fish, they can be referred to the genus *Otodistomum*, and are probably immature specimens of *O. cestoides*. This is the conclusion reached by Ohdner (16).

FAMILY GORGODERIDAE

Lepidophylum steenstrupi Ohdner, 1902

(Fig. 21)

HOST: *Annahrichas lupus* (wolf fish), from the urinary bladder.

The collection contains five specimens, only one of which harbours eggs. The body of this form is flattened, sac-shaped, with a broadly rounded posterior end from which it tapers to the anterior end. It is widest at the level of the testes. The cuticula is covered with fine spines. The oral

sucker is terminal and subspherical in outline. There is a large muscular pharynx, an oesophagus of about the same length, and two intestinal caeca that extend to slightly more than half-way down the length of the body. The acetabulum is slightly smaller than the oral sucker and is situated just anterior to the middle of the body. The ovary is somewhat lobed, and is situated just posterior to the acetabulum. The testes are deeply lobed, situated one on either side of the mid-line, with their anterior margins just below the level of the ovary. The vitellaria consist of a few follicles arranged in two groups, between, and slightly anterior to, the testes. The uterus passes back to the posterior tip of the body. The genital pore is situated near the right margin of the body, on a level a little below the intestinal bifurcation. The elongate cirrus pouch reaches back to the anterior margin of the testes. The mature specimen shows the following measurements: length 1.5 mm., width 0.9 mm., oral sucker 0.17 mm. in diameter, pharynx 0.126 mm. long, acetabulum 0.15 mm. in diameter, eggs 0.04 by 0.02 mm.

Phyllodistomum folium van Olfers, 1816

HOST: *Esox lucius* (pike), from the urinary bladder.

The collection contains a single specimen with the posterior part of the body completely filled with eggs, which obscure the internal anatomy. The specimen measures 1.21 by 0.74 mm. The oral sucker is 0.130 mm. in diameter, the ventral sucker being somewhat larger measures 0.163 mm. in diameter. The eggs measure 0.032 by 0.016 mm.

As Stafford points out, his specimen differs from the description of *P. folium* in the size of the two suckers. Furthermore, the size of the eggs is smaller in his specimen. However, it does resemble *P. folium* in the general size and shape of the body and was recovered from the same host. Until further specimens from the same host can be studied, it is thought best to consider it as *P. folium*.

FAMILY HETEROPHYIDAE

Cryptogonimus diaphanus (Stafford, 1904)

(Fig. 24)

(*Protenteron diaphanum*, of Stafford)

HOST: *Ambloplites rupestris* (rock bass), from the intestine.

Stafford described a trematode from the intestine of the rock bass, as a new species, and referred it to the new genus *Protenteron*. His collection does not contain any specimens that exactly fit his description. However, there is one specimen from the rock bass that was collected in October, 1904, and therefore after his paper was published, which is very probably the same species. Stafford's description is as follows:

"A living specimen measured 1.54 × 0.385. Broadest at the middle, narrowing behind. Mouth-sucker terminal, 0.186. Ventral sucker small 0.062 (Stafford's figure of 0.62 is obviously a misprint), situated 0.57 from the anterior end. Skin with fine spines. Pre-pharynx longer than either

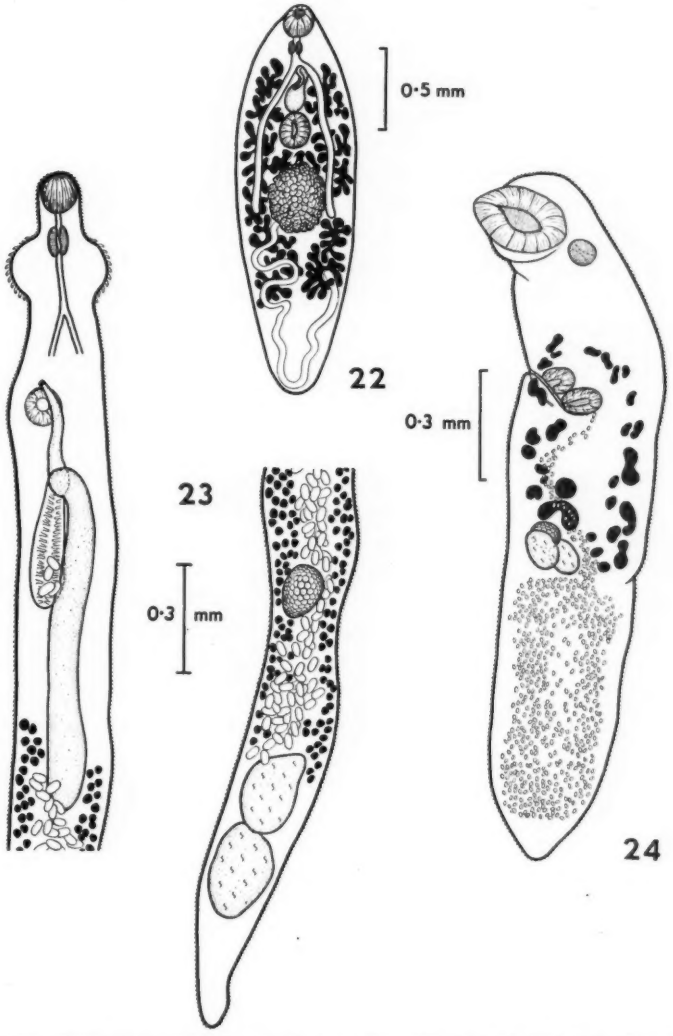

FIG. 22. *Centrovarium lobotes, ventral view.* FIG. 23. *Deropristis inflata, dorsal view.* FIG. 24. *Cryptogonimus diaphanus.*

pharynx or oesophagus. Short caeca diverging and falling short of ventral sucker or scarcely passing it. Black eyespots lateral from pharynx. Testes obliquely side by side in middle of post-acetabular body. Ovary a little in front of left testis. Uterus reaching to posterior end—older eggs in the folds to the left. Egg 0.022 by 0.011 (again Stafford's figure of 0.22 for the egg length is obviously a misprint). Genital sucker close in front of ventral

sucker. Penis apparatus reaching behind to the ovary. Vitellaria lateral, short, from the forking of the intestine to near the ovary."

The specimen in the collection is poorly stained, and as the body is twisted it is difficult to make out all the organs. It measures 1.9 by 0.4 mm. The cuticula is covered with fine spines. Eyespots are present. While the pre-pharynx cannot be made out, the pharynx is some distance from the oral sucker, suggesting that the pre-pharynx is fairly long. The oral sucker is about three times the size of the acetabulum. The testes are obliquely side by side in the middle of the post-acetabular body, and the ovary appears to be in front of the left testis although this is difficult to make out as the specimen is twisted laterally. The vitellaria extend from the intestinal bifurcation to the level of the ovary. The uterus reaches to the posterior tip of the body. The egg size is the same as that given by Stafford, 0.02 by 0.01 mm. The penis apparatus mentioned by Stafford cannot be seen, nor are the intestinal caeca visible. However, the genital pore is surrounded by a muscular sucker, a character Stafford failed to observe.

Cryptogonimus diaphanus is undoubtedly closely related to *C. chyli*, which occurs in the same host. It resembles it in the spiny cuticula, the presence of eyespots, the distribution of the genital organs, and in the extent of the intestinal caeca. Furthermore, the muscular genital atrium-like structure is very much like that found in *C. chyli*. It differs from *C. chyli* in the larger size, the smaller genital organs, and non-lobate ovary, and in the presence of an elongate pre-pharynx.

The specimen of *Cryptogonimus* at hand shows the following measurements: length 1.9 mm., width 0.4 mm., oral sucker 0.22 mm. by 0.14 mm., testes 0.09 and 0.10 mm. in length, the right and left, respectively, ovary somewhat smaller than the testes, eggs 0.2 by 0.1 mm. The exact measurements of the acetabulum could not be obtained. However, it appeared to be slightly less than one-third the size of the oral sucker. The pharynx is about the same size as the acetabulum.

Centrovarium lobotes (McCallum, 1895)

(Fig. 22)

HOST: *Esox lucius* (pike), *Stizostedion vitreum* (doré), from the stomach.

Stafford created the genus *Centrovarium* to include *Distomum lobotes* McCallum. More recently Van Cleave and Mueller (21) made a careful study of this form and placed it in the family Heterophyiidae.

The collection contains four specimens, three from the pike and one from the doré. The body of these parasites is oval with rounded ends; the anterior body region is usually the broader. They range from 1.6 to 2.3 mm. in length. The terminal oral sucker is spherical in outline. There is a short pre-pharynx, a small pharynx, and an oesophagus about the same length as

the pharynx. The intestinal caeca extend about half-way down the body length. The acetabulum is situated slightly less than a third of the body length from the anterior tip. The large rosette-shaped ovary is situated in the middle of the body. The testes are arranged one on each side of the body, a short distance posterior to the ovary. The uterus passes back to the posterior tip of the body. The genital pore lies on the mid-line, anterior to the acetabulum. The vitellaria extend from the intestinal bifurcation to within a short distance posterior to the ovary. The follicles are greatly branched and usually meet dorsally at the mid-line. The prominent cirrus sac may extend posterior to the acetabulum; the eggs measure 0.03 by 0.13 mm.

FAMILY ACANTHOCOLPIDAE

Deropristis inflata Molin, 1859

(Fig. 23)

HOST: *Anguilla chryspa* (eel), from the intestine.

Stafford reported two species of *Deropristis* from Canadian fishes. However, the collection contains only three specimens of *D. inflata*. Ward (22, pp. 509-521) doubts Stafford's record of *D. hispidus* from the sturgeon. The specimens in the collection show his record of *D. inflata* to be accurate.

The specimens at hand are rather on the large side, ranging from 3.4 to 3.7 mm. in length. They are long narrow forms with an expanded collar bearing a row of large spines near the anterior tip. The cuticula is covered with smaller spines throughout. The small oral sucker is spherical. There is an obvious pre-pharynx and a medium-sized pharynx which is somewhat shorter than the diameter of the acetabulum. The oesophagus is longer than the pharynx and forks into the narrow intestinal caeca a considerable distance anterior to the acetabulum. The intestinal caeca extend to within a short distance of the posterior tip. The acetabulum is situated about a fifth of the distance from the anterior tip. It is spherical, and about the same size as the oral sucker. The oval testes are in tandem arrangement near the posterior tip. The oval ovary lies about half-way between the posterior tip of the cirrus sac and the anterior testis. The uterus passes back to the anterior margin of the anterior testis. The genital pore is situated immediately anterior to the acetabulum, on the mid-line. The cirrus pouch extends back to the middle of the body. The vitellaria extend laterally from the posterior margin of the cirrus pouch to the anterior testis. The measurements of the best preserved specimen are as follows: length 3.4 mm., width 0.26 mm., oral sucker 0.10 mm. in diameter, pharynx 0.08 mm. long, acetabulum 0.10 mm. in diameter, ovary 0.2 by 0.09 mm., anterior testis 0.22 by 0.15 mm., posterior testis 0.26 by 0.16 mm., cirrus pouch 0.97 mm. long, eggs 0.045 by 0.024 mm.

Stephanostomum sp.

HOSTS: *Hemitripterus americanus* (sea raven)
Cryptocanthodes maculatus (wrymouth)
Lycodes sp., from the intestine.

The specimens are all somewhat faded and it is impossible to count the circum-oral spines accurately. The specimens from the sea raven range from 2.2 to 2.5 mm. in length, the specimen from the wrymouth measures 1.8 mm., and that from *Lycodes* 2.7 mm. in length. The specimens from the sea raven appear to have from 45 to 50 spines arranged in two rows, the spines of the anterior row being smaller. In general morphology these specimens approach *Stephanostomum baccatus* Nicoll, 1907, and together with the character of the circum-oral spines they show a great resemblance to that species. The specimen from *Lycodes* appears to have comparatively small spines around the oral opening, although in general appearance it also resembles *S. baccatus*.

References

1. HOPKINS, S. H. Illinois Biol. Monogr. 13 (2). 1934.
2. KUKENTHAL, W. and KRUMBACH, T. Handbuch der Zoologie. *Chapter by* O. Fuhrmann. 2 : 33–140. 1928.
3. LEBOUR, M. Rept. Northumberland Sea Fisheries, 3 : 3-47. 1908.
4. LINTON, E. U.S. Fish Comm. for 1899, 19 : 267-304. 1900.
5. LINTON, E. U.S. Fish Comm. for 1899, 19 : 405-492. 1901.
6. LOOSS, A. Zentr. Bakt. Parasitenk. 29 : 595-661. 1901.
7. LOOSS, A. Zool. Anz. 31 : 585-620. 1907.
8. LÜHE, M. Susswasserfauna Deutsch. Heft. 17 : 1–217. 1909.
9. MILLER, M. J. Can. J. Research, D, 18 : 423-434. 1940.
10. MANTER, H. W. Illinois Biol. Monogr. 10 (2). 1926.
11. NICOLL, W. Ann. Mag. Nat. Hist. Ser. 7, 19 : 66-94. 1907.
12. OHDNER, T. Zentr. Bakt. Parasitenk. 31 : 152-162. 1902.
13. OHDNER, T. Fauna Arctica, 4 : 291-372. 1905.
14. OHDNER, T. Zool. Anz. 37 : 237-253. 1911.
15. OHDNER, T. Zool. Anz. 38 : 97-117. 1911.
16. OHDNER, T. Zool. Anz. 38 : 513-531. 1911.
17. POCHE, F. Arch. Naturgesch. Abt. A, 2 : 112–244. 1926.
18. PRICE, E. W. Smithsonian Inst. Pub. Misc. Collections, 91 : 1-8. 1934.
19. STAFFORD, J. Zool. Anz. 27 : 481-495. 1904.
20. STILES, C. H. and HASSALL, A. Bull. U.S. Hyg. Lab. 37. 1908.
21. VAN CLEAVE, H. and MUELLER, J. Roosevelt Wild Life Ann. 3 : 161-334. 1934.
22. WARD, H. B. Livro Jub. Prof. L. Travassos. Rio de Janeiro. 1938.

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